EVELLYN CLAUDIA WIETZIKOSKI

ESTUDO COMPARATIVO DO COMPORTAMENTO IPSIVERSIVO E CONTRAVERSIVO NOS MODELOS DA 6-OHDA E MPTP DA DOENÇA DE PARKINSON

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

Orientador: Prof. Dr. Cláudio da Cunha

CURITIBA
Dezembro, 2006

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

"Sei que meu trabalho é uma gota no oceano,

mas sem ele, o oceano seria menor".

Madre Teresa de Calcutá

DEDICO este trabalho ao meu namorado, Ricardo Andrei Lovato; minha mãe, Idalina Menossi Wietzikoski; meu pai, Luiz Wietzikoski; minha irmã, Samantha Wietzikoski; meu cunhado, Humberto Itiro Sato; e ao mais novo membro da família, meu sobrinho, Henri Gabriel Sato.

Amo todos vocês,

Muito obrigada por vocês me apoiarem e confiarem em mim sempre.

ESTA VITÓRIA É NOSSA!!!

AGRADECIMENTOS

Ao Prof. Dr. Cláudio da Cunha, por quem tenho imensa admiração, sou muito grata por seus ensinamentos, por ter despertado em mim o interesse pela Ciência e tenho certeza de que tenho muita sorte por ser meu orientador. Você é um exemplo de pessoa e pesquisador. Muito obrigada!

Aos meus pais, por estarem comigo em mais esta importante etapa da minha vida. Vocês são os meus alicerces, agradeço sinceramente por todos os ensinamentos. Vocês torceram, choraram, riram, viveram todos os momentos tristes e felizes me apoiando, e fizeram eu me superar a cada dia. Vocês são muito importantes para mim. Amo vocês incondicionalmente.

Ricardo, meu amorzinho, você sempre me incentivou e cuidou de mim durante todos estes anos, esta força foi e continua sendo muito importante. Você faz parte da minha vida, obrigada pela paciência, compreensão, e é claro por todo seu amor. Te amo!

Á minha querida irmã, Samantha, o que vou dizer, somos cúmplices, você sabe que sou sua maior fã, você é um exemplo de coragem, determinação e vitória. Amo muito você.

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.

Á Prof^{a}. Dr^{a}. Débora Hipólide por sua colaboração e ensinamentos dados sobre a autoradiografia.

Aos amigos de laboratório que vou lembrar por toda minha vida, Marcelinho, Léa, Edmar, Lucélia, Mariza, Patrícia, Scheila, Michele, Jeff, Vinícius, Suelen, Nadia, Fernanda, Jaqueline e Inara pelo incentivo, ajuda com experimentos, troca de informações, aprendizado e pelo companheirismo.

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

Aos grandes amigos Ângela e Marcelo, vocês são extraordinários, dedicados e extremamente competentes. Também agradeço vocês por me hospedarem em sua casa em São Paulo e ainda pelos inúmeros favores realizados. Muito obrigada!

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, e minha amiga, 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.

Ás secretárias do Departamento de Farmacologia, Alessandra e Cristiane, por todo trabalho e dedicação aos alunos da pós-graduação.

Á CAPES pelo apoio financeiro.

Agradeço todos vocês.
Muito obrigada!!!

SUMÁRIO

LISTA DE ABREVIATURAS

5-HT	-	Serotonina
6-OHDA	-	6-hidroxidopamina
ATP	-	Adenosina trifosfato
ATV	-	Área tegmental ventral
DA	-	Dopamina
DOPAC	-	Ácido 3,4-dihidroxifenilacético
DP	-	Doença de Parkinson
FPM	-	Feixe prosencefálico medial
GABA	-	Ácido gama-aminobutírico
GPe	-	Globo pálido externo
GPi	-	Globo pálido interno
HPLC	-	Cromatografia de fase líquida de alta pressão
HVA	-	Ácido homovanílico
MAO-B	-	Monoamina oxidase B
$MPDP^+$	-	Diidropiridina
MPP^+	-	1-metil-4-fenilpiridina
MPTP	-	1-metil-4-fenil-1,2,3,6-tetrahidropiridina
NA	-	Noradrenalina
s.c.	-	Subcutâneo
SHAM	-	O mesmo que simulado, falsamente lesado
SN	-	Substância negra
SNC	-	Sistema nervoso central
SNc	-	Substância Negra parte compacta
SNr	-	Substância negra parte reticulada
TH	-	Tirosina Hidroxilase

LISTA DE FIGURAS

RESUMO

O comportamento rotatório em animais é uma ferramenta útil para testar drogas com ação sobre o sistema dopaminérgico. A infusão unilateral de 6-hidroxidopamina (6-OHDA) no feixe prosencefálico medial (FPM) de ratos provoca morte de todos os neurônios dopaminérgicos da substância negra parte compacta (SNc), mimetizando o que ocorre em uma fase adiantada da doença de Parkinson (DP). Esses animais quando desafiados com apomorfina apresentam comportamento rotatório contralateral a lesão, por outro lado, o desafio com anfetamina causa rotações ipsilaterais. Entretanto, a administração intra-nigral de 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) na SNc dos ratos ocasiona morte parcial de neurônios dopaminérgicos, reproduzindo o estágio inicial da DP. Esses animais, ao contrário dos lesados com 6-OHDA, apresentam rotações ipsilaterais após o desafio tanto com apomorfina quanto com anfetamina. Estes dois modelos animais da DP foram utilizados neste trabalho para estudar o comportamento rotatório após o desafio com apomorfina e anfetamina. No experimento 1 foi proposto validar o modelo animal de lesão com MPTP para o screening de drogas com potencial efeito para tratar os prejuízos motores em uma fase inicial da DP. Já no experimento 2, a proposta foi tentar explicar qual é o fator crítico para o sentido das rotações. Os resultados deste estudo sugerem que existem diferenças quanto ao mecanismo das neurotoxinas MPTP e 6-OHDA sobre o comportamento rotatório dos animais, e ainda, que essas diferenças parecem estar relacionadas com o tamanho/magnitude da lesão provocada pelas neurotoxinas. Uma lesão grande causada pela 6-OHDA aumenta a expressão de receptores D2 no estriado, porém a lesão parcial produzida pelo MPTP não é capaz de gerar supersensibilização. Portanto, o comportamento rotatório ipsilateral pode estar reproduzindo o que ocorre em pacientes nos estágios iniciais com DP, gerando um modelo válido para screening de drogas antiparkinsonianas.

ABSTRACT

The turning behavior in animals is an useful tool to test drugs with action on the dopaminergic system. The unilateral infusion of 6-hidroxidopamine (6-OHDA) into the medial forebrain bundle (MFB) of rats caused the death of all dopaminergic neurons of the substantia nigra, pars compacta (SNc), mimicking what happens in the early phase of Parkinson's disease (PD). These animals when challenged with apomorphine present contralateral turns, on the other hand, the challenge with amphetamine cause ipsilateral turns. However, the administration of the 1-methyl-4-fenil-1,2,3,6-tetrahidropiridium (MPTP) into the SNc of the rats caused the partial lost of the dopaminergic neurons, resembling the beginning of PD. Differently of 6-OHDA, MPTP causes ipsilateral turns after the challenge with both apomorphine or amphetamine. These two animal models of PD were used in this work to study the turning behavior after the challenge with apomorphine and amphetamine. In the experiment 1 the purpose was to validate the animal model of MPTP lesion for the screening of drugs with potential effect to treat the motor impairment in the early stage of PD. In the experiment 2, the goal was to explain which is the critical factor for the direction of the rotations.

The results of this study suggest that the mechanisms of MPTP and 6-OHDA lesions are different, what may influence on the turning behavior of the animals. Actualy, those differences seem to be related with the size of the lesions caused by the neurotoxines. The larger lesion caused by the 6-OHDA increases the expression of D2 receptor, while the partial lesion produced by MPTP is not capable to generate supersensitization. Therefore, the ipsilateral turning behavior can reproduce what happens in patients at the initial stage of PD, generating a valid model for the screening of antiparkinsonian drugs.

1 INTRODUÇÃO

A doença de Parkinson (DP) é caracterizada por uma degeneração de neurônios dopaminérgicos na substância negra parte compacta (SNc) (BLUM *et al.*, 2001; FLINT, 2001; YOUDIM e RIEDERER, 1997) e pela presença de inclusões esféricas citoplasmáticas denominados corpos de Lewy, que consistem de uma densa camada granular cercada por um halo que se propaga em filamentos (FLINT, 2001; FORNO, 1996). Os corpos de Lewy presentes na substância negra (SN) e no *locus coeruleus* não são patognomônicos da DP, porém, representam marcadores anatomopatológicos. No interior dos corpos de Lewy, encontra-se a proteína alfa-sinucleína, além de outras que contribuem significativamente para o processo de degeneração dos neurônios dopaminérgicos do encéfalo (MENESES e TEIVE, 2003). A presença de corpos de Lewy não é restrita ao sistema nervoso central, uma vez que eles também são observados no sistema nervoso periférico de pacientes parkinsonianos (VANDERHAEGHEN *et al.,*1970) e em outras desordens degenerativas, tais como, doença de corpos de Lewy e na esclerose lateral amiotrófica (BLUM *et al.*, 2001).

Em pacientes parkinsonianos, ocorre uma perda de dopamina (DA) e seus metabólitos: ácido homovanílico (HVA) e ácido diidroxifenilacético (DOPAC), e do transportador de dopamina no estriado e na SNc (BEAL, 2001). Além do decréscimo de DA na via nigroestriatal, pode ocorrer também degeneração de neurônios dopaminérgicos na área tegmental ventral (ATV) (AGID *et al.*, 1990), redução de noradrenalina nos neurônios do *locus coeruleus*, perda de serotonina (5-HT) nos núcleos da rafe e redução de acetilcolina no núcleo basal de Meynert (CANDY *et al.*, 1983). A perda de neurônios nigrais ocorre principalmente em uma área localizada lateralmente na porção ventral da SNc (DEL TREDICI *et al.,* 2002; FEARNLEY e LEES, 1991; GOTO *et al.*, 1989; HIRSCH *et al.,* 1988).

Durante o processo fisiológico do envelhecimento, ocorre morte dos neurônios dopaminérgicos, em torno de 5% a cada década (BRENNER e ARNDT, 2004; BRAAK *et al.*, 2003; FEARNLEY e LEES, 1991; McGEER *et al.*, 1989), porém na DP, esta perda ocorre de forma rápida e progressiva, podendo ocasionar até 45% de morte neuronal por década (DUNNET e BJÖRKLUND,

1994). Os sintomas motores surgem quando ocorre uma diminuição entre 70 a 80% da DA estriatal e perda de mais de 50% de neurônios dopaminérgicos da SNc (DEL TREDICI *et al.,* 2002; FEARNLEY e LEES, 1991; McGEER *et al.,* 1989). A progressão dos sintomas ocorre lentamente, porém a velocidade com que a progressão se desenvolve varia de indivíduo para indivíduo. As manifestações motoras incluem: acinesia, bradicinesia, tremor em repouso, rigidez muscular, distúrbios do equilíbrio e da marcha, porém evidências clínicas e experimentais indicam que esta desordem neurodegenerativa também causa déficits cognitivos (Da CUNHA *et al.*, 2006; FERRO *et al.*, 2005; Da CUNHA *et al.*, 2003; MYOSHI *et al.*, 2002), depressão, alterações do sono e distúrbios do sistema nervoso autônomo (FLINT, 2001).

A prevalência da DP tem sido estimada entre 85 e 187 casos por 100.000 pessoas ou 1% da população com idade superior a 55 anos. O início do quadro clínico ocorre geralmente entre 50 e 70 anos de idade, porém, podem-se encontrar pacientes com início da doença mais precoce, antes dos 40 anos e, até mesmo, abaixo dos 21 anos de idade (MENESES e TEIVE, 2003).

A descoberta das doenças de Parkinson e Huntington como desordens causadas respectivamente pelo neurodegeneração da SNc e do estriado, provêem evidências de que essas estruturas possuem um importante papel no controle motor. Sabe-se que o disparo dos neurônios do córtex motor é controlado por uma via córtex - estriado - globo pálido - tálamo - córtex. Este circuito é controlado através de neurônios da via nigroestriatal que ativam a via direta através dos receptores dopaminérgicos D1 (inibida na DP) ou inibem a via indireta através dos receptores dopaminérgicos D2 (exarcebada na DP) (ALEXANDER e CRUTCHER, 1990). Além do seu papel no controle de motor, a liberação de dopamina através dos neurônios nigroestriatais é crítica para plasticidade sináptica necessária no processo de aprendizagem e memória (Da CUNHA *et al.,* 2006; BRAGA *et al*., 2005; BELLISSIMO *et al*., 2004; Da CUNHA *et al.*, 2003, 2002, 2001; MIYOSHI *et al*., 2002; GEVAERD *et al*., 2001a, b).

Os sintomas clínicos da DP mais predominantes são as desabilidades motoras, fato que contribui para uma visão prevalente de que os gânglios da base estão envolvidos principalmente com o controle das funções motoras

(HEIKKILA *et al.,* 1989). Dentre as estruturas que compõem os gânglios da base, a substância negra encontra-se localizada no mesencéfalo, e recebe este nome devido à presença de neuromelanina, um pigmento presente em neurônios dopaminérgicos da SNc (BOGERTS, 1981; GRAHAN, 1978). Apresenta-se dividida morfologicamente em duas partes: a parte reticulada (SNr), que possui na sua maioria neurônios GABAérgicos; e a SNc que está localizada acima da SNr e é formada principalmente por corpos celulares de neurônios dopaminérgicos que projetam seus axônios para o núcleo caudado e putâmen, formando a via nigroestriatal (LENT, 2001).

A via dopaminérgica nigroestriatal é composta pelo grupo de células A9 que fica situado na SNc. Os axônios destes neurônios percorrem ao longo do feixe prosencefálico medial (FPM) e terminam no estriado dorsal. A maior parte da via nigroestriatal projeta fibras ipsilateralmente, sendo escassas projeções contralaterais. O complexo estriatal pode ser dividido em uma parte dorsal (núcleo caudado e putâmen) e uma parte ventral (incluindo o núcleo accumbens). No cérebro humano, o núcleo caudado e o putâmen são separados anatomicamente pela cápsula interna. Em contraste, no cérebro do rato não ocorre esta separação anatômica, e então, esta estrutura cerebral é chamado de complexo caudado-putâmen. Por causa da extensa perda de neurônios dopaminérgicos da área A9 na DP, ocorre um declínio da DA estriatal conduzindo aos prejuízos motores. Além da SNc, há também significante degeneração nos grupos de células dopaminérgicas das áreas A8 (área retrorubral que projeta para o putâmen ventrocaudal) e A10 (ATV que projeta para o núcleo accumbens) (DEUMENS *et al.*, 2002; JELLINGER,1987).

Do estriado partem duas vias de saída para o tálamo, a direta e a indireta. Na via direta, os neurônios que saem do estriado, liberam o neurotransmissor inibitório ácido gama-aminobutírico (GABA) e o neuropeptídeo substância P que vão inibir os neurônios GABAérgicos no globo pálido interno (GPi) e na SNr, liberando desta forma o movimento. Na via indireta, o estriado projeta neurônios contendo GABA e encefalinas que inibirão o globo pálido externo (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, impedindo o movimento (figura 1) (WICHMANN e DeLONG, 2006; ALEXANDER *et al.*, 1986).

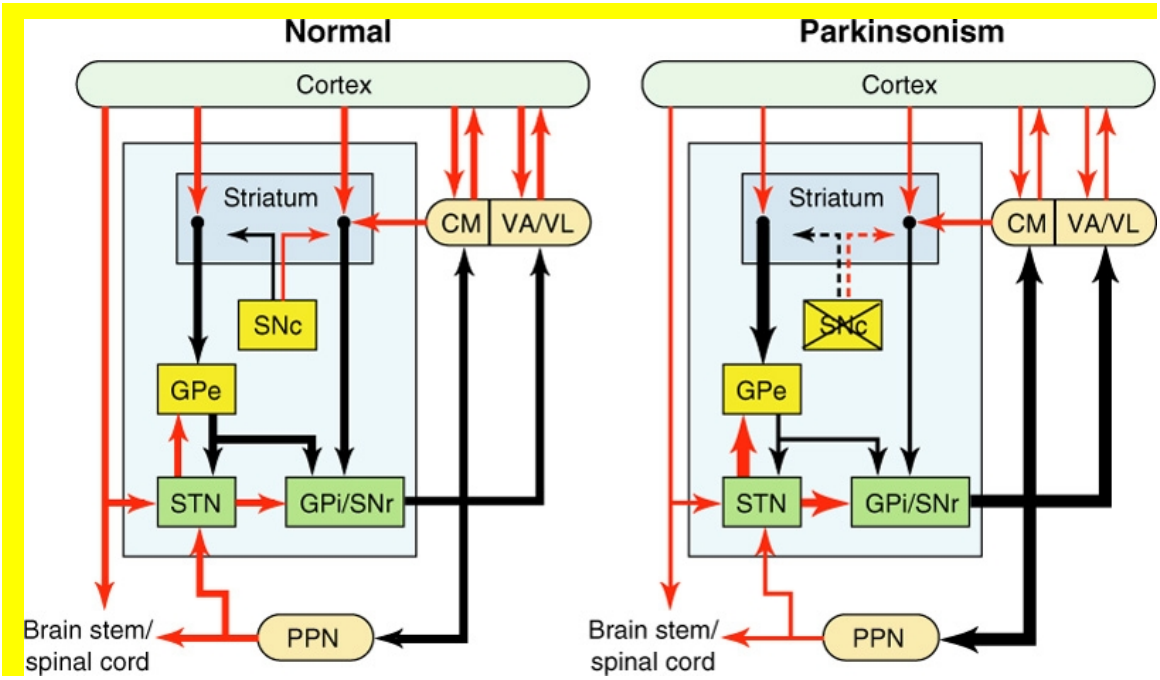

Figura 1: Diagrama simplificado demonstrando as conexões anatômicas dentro do circuito dos gânglios da base, e as mudanças na atividade dos núcleos dos gânglios basais associadas com o desenvolvimento de parkinsonismo. GPe, globo pálido externo; STN, núcleo subtalâmico; GPi, globo pálido interno; SNr, substância negra parte reticulada; SNc, substância negra parte compacta; PPN, núcleo pedúnculo pontino; CM, núcleo centro-mediano do tálamo; VA, núcleo ventro-anterior do tálamo; VL, núcleo ventrolateral do tálamo. Setas vermelhas representam as conexões excitatórias, setas pretas identificam as conexões inibitórias (GABAérgicas). Mudanças na largura das setas indicam mudanças de atividade (WICHMANN e DeLONG in SIEGEL *et al.,* 2006).

De acordo com Fallon e Loughlin (1985), os neurônios da SN podem ser quimicamente classificados em dopaminérgicos e não-dopaminérgicos. Os primeiros são amplamente dispersos por toda SN e adjacências podendo ser identificados pela combinação de certas características, como o seu tamanho, que varia de 15 a 45 μm, por sua forma poligonal, fusiforme ou trigonal, pela presença de melanina e de tirosina-hidroxilase, identificadas por técnicas histológicas. Os neurônios não-dopaminérgicos, em geral, possuem forma de espícula, de neuroglia ou são esféricos, e possuem diâmetros de 8 a 15 μm.

As funções da SN estão relacionadas com a atividade motora, funções sensoriais, límbicas, neuroendócrinas, e motivacionais. Disfunções da SN implicam na etiologia de transtornos motores, afetivos e neuroendócrinos, como o mal de Parkinson, a Coréia de Huntington, discinesia tardia, esquizofrenia, transtorno bipolar (FALLON e LOUGHLIN, 1985).

As desordens neurológicas em humanos podem ser reproduzidas em modelos animais utilizando procedimentos que reproduzem os eventos

patológicos e comportamentais. Além de prover uma ferramenta indispensável para pesquisa básica, os modelos animais de desordens humanas permitem investigar estratégias terapêuticas como condições prévias para se testar novas drogas em pacientes.

Modelos experimentais da DP que reproduzem a desnervação dopaminérgica têm sido desenvolvidos para estudar a patofisiologia da doença e para analisar a eficácia de novas terapêuticas. A DP é uma doença humana, e não se manifesta espontaneamente em animais, é somente observada através da administração de agentes neurotóxicos que interrompam ou destruam seletivamente o sistema catecolaminérgico, tais como a 6-hidroxidopamina (6-OHDA), a metanfetamina, a 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) (KAHLE *et al.*, 2002; GERLACH e RIEDERER, 1996). Alguns agentes químicos, como a rotenona, quando administrados sistemicamente podem induzir aspectos específicos da DP (HALBACH *et al.*, 2004; BETARBET *et al.*, 2002).

Animais com lesão da SNc pela toxina MPTP (LANGSTON *et al.*, 1983) são utilizados como um modelo da doença de Parkinson. A descoberta do MPTP aconteceu em 1982 quando um grupo de usuários de droga da Califórnia desenvolveu os sintomas severos de parkinsonismo. A investigação revelou que a síndrome foi causada pela administração de um análogo sintético da heroína que tinha sido contaminado por um subproduto, o MPTP (BEAL, 2001).

Existem diferenças quanto à suscetibilidade ao MPTP, e ocorrem por fatores como a idade, espécie e fatores genéticos. Portanto para se obter um modelo animal da DP, através do uso do MPTP, devem ser considerados o método de administração e a espécie animal a ser utilizada. Os animais mais usados para estudos do MPTP são camundongos e macacos. O MPTP, por ser um composto altamente lipofílico, após sua administração sistêmica atravessa facilmente a barreira hematoencefálica, atingindo rapidamente o sistema nervoso central (EMBORG, 2004; MENESES e TEIVE, 2003). O MPTP, composto pré-tóxico, é convertido pela monoamino oxidase tipo B (MAO-B), dentro das células gliais e dos neurônios serotoninérgicos, para diidropiridina ($MPDP^+$), o qual posteriormente é metabolizado para o metabólito ativo 1-metil-4-fenilpiridina (MPP^+) (BEZARD *et al.*, 1999; NICKLAS *et al.,*1985). Como o

MPP^+ é uma molécula polar, não pode entrar livremente no espaço intracelular, porém, através do sistema de recaptação da DA, é transportado para células dopaminérgicas, onde é acumulado. Dentro dos neurônios, o MPP^+ pode seguir por três rotas (figura 2): (1) Pode se ligar ao transportador vesicular da monoamina 2 (VMAT-2), que transloca o MPP^+ para dentro das vesículas sinaptossômicas. Esse seqüestro do MPP^+ ocorre para proteger as células da neurodegeneração induzida por MPTP, o que previne seu acesso à mitocôndria. (2) Pode se concentrar dentro da mitocôndria; quando isso ocorre, o MPP^+ bloqueia o complexo I, que interrompe a transferência de elétrons para a ubiquinona. Essa perturbação aumenta a produção de espécies reativas de oxigênio e diminui a síntese de adenosina trifosfato (ATP). (3) Pode permanecer no citosol, interagindo com enzimas citosólicas, especialmente as que são carregadas negativamente (DAUER e PRZEDBORSKI, 2003). O MPP^+ é então captado pela mitocôndria, onde inibe o complexo I da cadeia respiratória mitocondrial, bloqueando a fosforilização oxidativa e a produção de ATP, levando assim ao processo de morte celular (SMEYNE e JACKSON-LEWIS, 2005; EMBORG, 2004; LANGSTON, 1996). A literatura não relata qualquer estudo que sugere que o metabólito tóxico do MPTP, o MPP+ que é captado pelos corpos ou dentritos de neurônios dopaminérgicos, também seja captado por axônios.

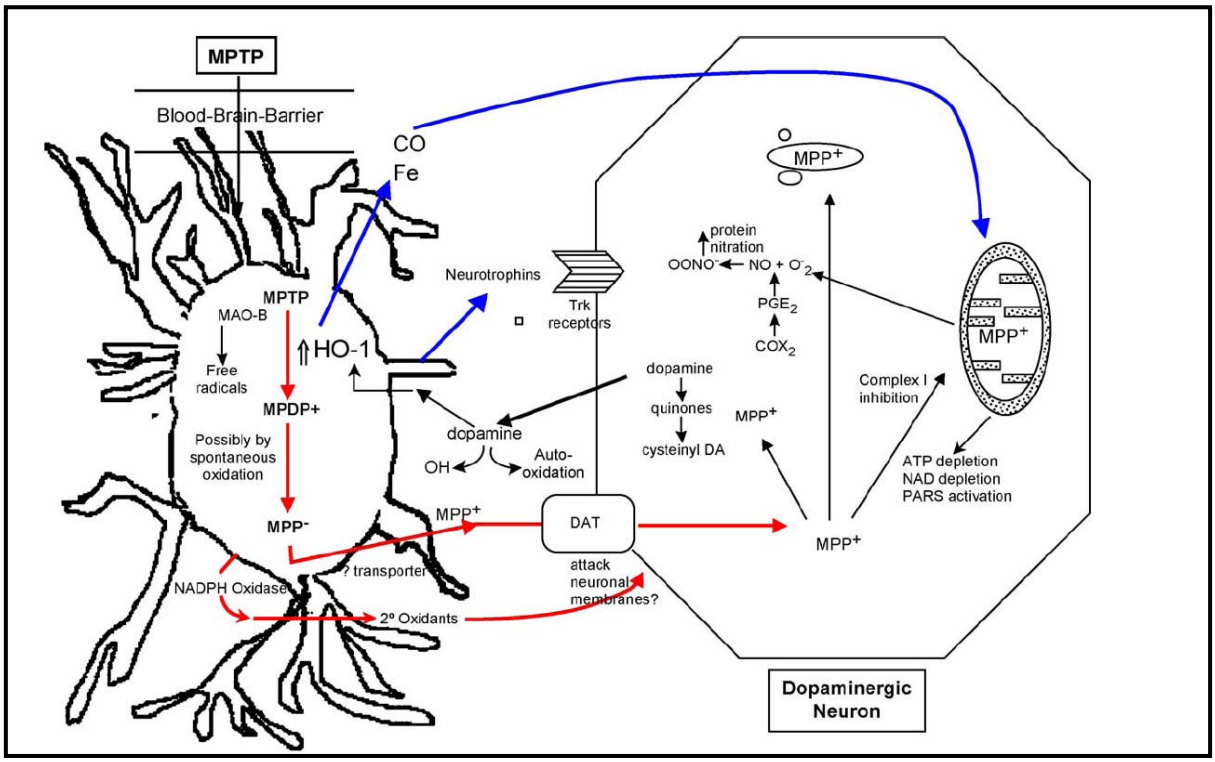

Figura 2: Representação esquemática do mecanismo de ação de MPTP no sistema nigrostriatal. Setas vermelhas representam o papel inicial (tóxico) das células da glia. Setas azuis representam o segundo papel (neuroprotetor) das células gliais (SMEYNE e JACKSON-LEWIS, 2005).

Ratos são menos sensíveis ao MPTP (GIOVANNI *et al.*, 1994a, 1994b) não sendo efetiva a administração sistêmica, sendo necessário, portanto a infusão intracerebral do MPTP ou do seu metabólito MPP^+ (STAAL *et al.*, 2000; SONSALLA *et al.,* 1992; STOREY *et al.*, 1992). A administração intranigral de MPTP na SNc dos ratos ocasiona perdas específicas de DA no estriado e córtex pré-frontal. Esta substância apresenta uma grande afinidade pelos neurônios da SNc, não afetando significativamente os demais neurônios dopaminérgicos (GANONG, 1995). Já se sabe que lesões bilaterais da SNc com MPTP produzem, em ratos, prejuízos na memória equivalentes aos observados na DP em humanos (Da CUNHA *et al.*, 2006; FERRO *et al.*, 2005; BRAGA *et al.*, 2005; MYIOSHI *et al.*, 2002; GEVAERD *et al.*, 2002b, 2001a; Da CUNHA *et al.*, 2001).

A 6-OHDA é uma das neurotoxinas mais comuns utilizadas experimentalmente em modelos de degeneração da SNc, tanto *in vitro* como também *in vivo* (BLUM, 2001). É incapaz de atravessar a barreira hematoencefálica, sendo necessária a administração diretamente na estrutura

cerebral que se deseja lesar. A injeção bilateral de 6-OHDA na SN ou em outras regiões cerebrais provoca uma elevada mortalidade neuronal, principalmente de neurônios catecolaminérgicos (FERRO *et al.*, 2005). É transportada para os neurônios catecolaminérgicos através de mecanismos de transporte específico (por exemplo: transportador de dopamina e o transportador de noradrenalina). Induz a produção de radicais livres como o peróxido de hidrogênio e o radical hidroxil, e é mais tóxica que o MPP^+ para o complexo I mitocondrial (figura 3) (BETARBET *et al.*, 2002; BLUM *et al.*, 2001; GLINKA *et al.*, 1997; GERLACH e RIEDERER, 1996; JONSSON e SACHS, 1975; THOENEN e TRANZER, 1968; UNGERSTEDT, 1968). As ações dos produtos de oxidação formados a partir desta neurotoxina são responsáveis pelos danos irreversíveis provocados aos neurônios catecolaminérgicos e sua subseqüente degeneração. Embora a 6-OHDA seja utilizada como uma neurotoxina seletiva, ela pode causar uma lesão não específica a qualquer tecido cerebral que contenham neurônios catecolaminérgicos (KONDOH *et al.*, 2005). Uma seletividade razoável para neurônios dopaminérgicos é alcançado através do pré-tratamento dos animais com desipramina, uma droga que bloqueia o transportador de noradrenalina, isto inibe a captação da 6-OHDA nos neurônios noradrenérgicos (DEUMENS *et al.*, 2002).

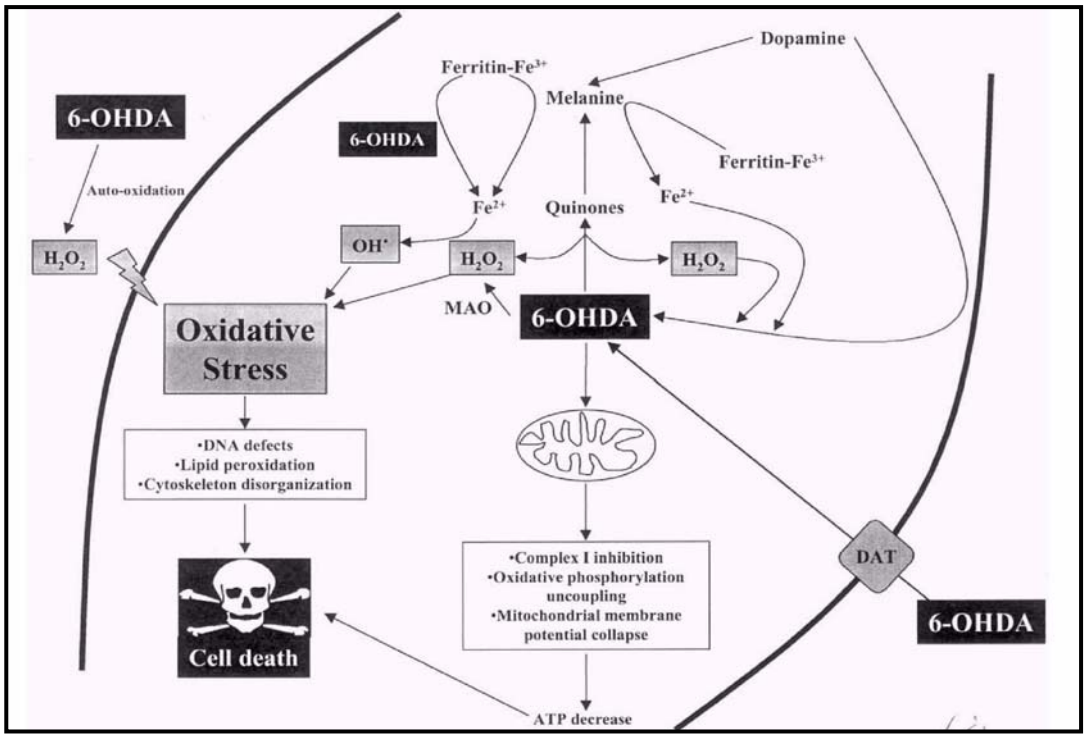

Figura 3: Mecanismo hipotético da toxicidade da 6-OHDA. A 6-OHDA pode induzir morte dos neurônios catecolaminérgicos por três mecanismos principais: geração de espécies reativas de oxigênio por auto-oxidação intra ou extracelular, formação de peróxido de hidrogênio pela atividade da MAO ou inibição da cadeia respiratória mitocondrial (BLUM, *et al,* 2001).

Animais lesados bilateralmente com 6-OHDA demonstram os sintomas motores parkinsonianos, entretanto, a lesão bilateral não é um modelo comum (FERRO *et al.*, 2005; CENCI *et al.*, 2002). A 6-OHDA é usualmente injetada unilateralmente, enquanto que o hemisfério intacto funciona como um controle interno, constituindo um modelo de hemiparkinsonismo, que é caracterizado por um comportamento motor assimétrico após a administração de drogas dopaminérgicas, devido a um desequilíbrio fisiológico entre o lado lesado e não-lesado (BETARBET *et al.*, 2002). A injeção unilateral de 6-OHDA na porção medial da SNc ou no FPM provoca a morte de todos os neurônios dopaminérgicos no mesencéfalo do lado aplicado e a diminuição de DA no lado correspondente do estriado, gerando um modelo válido de uma fase adiantada da DP com pronunciadas alterações motoras. Quando é administrada no FPM ocorre morte dos neurônios dopaminérgicos da SNc e da ATV devido ao transporte axonal retrógrado aos terminais dopaminérgicos estriatais (SCHWARTING e HUSTON, 1996; FALLON e LOUGHLIN, 1985; ZIGMOND, *et al.*, 1984). Esses animais apresentam comportamento rotatório contralateral

quando desafiados com agonistas D1/D2 da dopamina tais como a apomorfina e a bromocriptina. Drogas que induzem a liberação de DA, tais como a anfetamina, causam rotações ipsilaterais (METZ e WHISAW, 2002). Esse comportamento pode ser explicado pela hiperexpressão dos receptores dopaminérgicos na porção lesada do estriado, ou seja, as drogas agonistas terão seu efeito potencializado pela hipersensibilização dos receptores (GERLACH e RIEDERER, 1996; SCHWARTING *et al*., 1991).

O modelo rotatório de roedores, produzido pela lesão unilateral da via nigroestriatal, é uma ferramenta muito utilizada para testar drogas agonistas e antagonistas dopaminérgicos e ainda para estudos de problemas motores que mimetizam os sintomas da DP (GERLACH e RIEDERER, 1996). Os experimentos pioneiros foram realizados por Ungerstedt (1968) e Ungerstedt e Arbuthnott (1970) que relataram que ratos com lesões unilaterais induzidas por 6-OHDA demonstravam movimentos rotatórios espontâneos para o mesmo lado da lesão (ipsiversivo). Este comportamento rotatório foi observado durante as primeiras quatro semanas após a lesão. Drogas que agem liberando DA, ou bloqueando o transportador de dopamina, por exemplo, anfetamina, produz uma rotação ipsiversiva pronunciada devido a um aumento preferencial do conteúdo sináptico de dopamina no estriado intacto (SCHWARTING *et al*., 1991; ZETTERSTROM *et al*., 1986), em contraste, a administração de um agonista dopaminérgico pós-sináptico, como apomofina e L-dopa, induz rotação contraversiva devido à excitação dos receptores estriatais assimétricos (LANE *et al*., 2006). É citado na literatura que diferentes tamanhos (SCHWARTING & HUSTON, 1996) e locais de lesão (HIRSCHHORN *et al.*, 1983) podem influenciar a intensidade e o sentindo da rotação.

Em um estudo realizado por Kondo *et al.* (2004) para comparar a neurotoxicidade da 6-OHDA, MPP^+ e rotenona infundidos unilateralmente na SNc de ratos, obteve-se como resultado que a injeção de 6-OHDA causou uma maior perda de neurônios dopaminérgicos na SNc e depleção de DA no estriado, e o desafio com apomorfina induziu rotações contralaterais. Em contraste, o comportamento rotatório de animais lesados por MPP^+ causou rotações ipsilaterais, e não foi observado comportamento rotatório nos animais lesados com rotenona. Apesar de o MPP^+ e a rotenona causarem uma perda de neurônios dopaminérgicos dentro da SNc, a neurodegeneração estriatal foi

variável e menor do que a produzida pela 6-OHDA. Outros estudos (SINDHU *et al.*, 2006; SINDHU *et al.*, 2005) demonstraram que ratos Sprague-Dawley infundidos unilateralmente com rotenona na SNc comportaram-se rodando ipsilateralmente a lesão quando desafiados com apomorfina ou anfetamina, e ainda que a infusão no FPM de MPP^+ ocasionou rotações contralaterais após desafio com apomorfina.

Nesse modelo de rotação, a administração de apomorfina é o teste padrão da literatura para verificar a estimulação de receptores dopaminérgicos. A apomorfina foi o primeiro agonista dopaminérgico a ser usado na terapia da DP. Esta droga atua sobre os receptores dopaminérgicos D1 e D2 e em doses menores atua sobre o auto-receptor pré-sináptico inibindo o turnover de dopamina (DELEU *et al.*, 2002). A apomorfina é uma droga lipofílica administrada por via subcutânea devido ao metabolismo de primeira passagem, e provoca efeitos adversos quando administrada por via oral. É administrada junto com a levodopa ou em períodos “off” da levodopaterapia. O início do efeito da droga após a administração subcutânea varia de 5 a 20 min e dura até 1 hora em pacientes no início da doença (DELEU *et al.*, 2002). Náuseas e vômitos são os efeitos colaterais mais freqüentes da apomorfina. Problemas neuropsiquiátricos são observados em poucos pacientes ao longo do tratamento da DP (DELEU *et al.*, 2002).

O modelo de lesão da via nigroestriatal com 6-OHDA é utilizado para o estudo das alterações motoras da DP e ação de drogas antiparkinsonianas (LANE, *et al*., 2006; UNGESTEDT, 1970, 1968), já o modelo de lesão da via nigroestriatal com MPTP é utilizado para o estudo dos déficits cognitivos (Da CUNHA *et al.*, 2006; Da CUNHA *et al.*, 2003; MIYOSHI *et al.*, 2002). Não existem muitos estudos utilizando a neurotoxina MPTP para avaliar o comportamento motor na rotação.

Na literatura, para trabalhar com amostras mais homogêneas, muitos autores excluem de seus estudos animais que apresentam comportamento rotatório ipsilateral a lesão (MARIN *et al.,* 2006; LANE *et al.,* 2006; TAKEDA *et al.*, 2005; KONDOH *et al.*, 2005). Neste trabalho incluímos tanto animais que apresentaram comportamento rotatório ipsilateral como também os que apresentaram comportamento contralateral. Os poucos estudos utilizando o MPTP geram dúvidas se este modelo é válido para o estudo de alterações

motoras associadas à DP, por outro lado ele tem sido amplamente utilizado para estudos dos déficits cognitivos associados a esta doença. Para validá-lo nos estudos das alterações motoras, foram testados agonista dopaminérgico direto e indireto em várias doses sobre o comportamento rotatório dos animais com lesão unilateral por 6-OHDA ou MPTP. A 6-OHDA é citado na literatura como o modelo ouro para o teste de rotação após desafio com apomorfina ou anfetamina. Os animais lesados unilateralmente por MPTP apresentaram um comportamento rotatório ipsilateral dose-dependente após desafio com apomorfina ou anfetamina, validando o modelo para testar novas terapêuticas para a fase inicial da DP.

Entretanto, houve diferenças quanto ao comportamento rotatório produzido pelos animais lesados por 6-OHDA e MPTP. Para compreender o que determina a direção da rotação nos animais foram testadas várias hipóteses, como a localização do sítio de infusão da neurotoxina, o tamanho/magnitude produzido pela lesão e ainda, a expressão de receptores dopaminérgicos no estriado desnervado. Com a observação do comportamento dos ratos deste estudo, podem-se gerar conhecimentos que auxiliam na compreensão dos mecanismos fisiológicos que conduzem aos déficits motores e ainda entender qual é o limiar que separa os estágios inicias e tardios da DP.

2 OBJETIVOS

- Validar o modelo do comportamento rotatório produzido pela lesão com MPTP para estudo de drogas com efeitos sobre os prejuízos motores no estágio inicial da DP.
- Comparar o efeito de lesões em diferentes sítios da SNc induzidas pelas neurotoxinas 6-OHDA e MPTP sobre o comportamento rotatório em ratos.

EXPERIMENTO 1

COMPORTAMENTO ROTATÓRIO IPSILATERAL EM RATOS LESADOS POR MPTP COMO UM MODELO PARA A FASE INICIAL DA DOENÇA DE PARKINSON

Trabalho submetido para publicação na revista Britsh Journal Pharmacology, 2006.

Ipsilateral turning behaviour of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned rats as a model of early-stage Parkinson's disease

[1]Evellyn Claudia Wietzikoski, [1]Ivana Kouzmine, [1]Michele Lima Gregorio, [2]Marcelo Machado Ferro, [2]Joana Lea Meira Silveira, [3]Newton Sabino Canteras, [1]Maria Aparecida Barbato Frasão Vital, [*,1]Claudio Da Cunha

[1]Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Departamento de Farmacologia, UFPR, Curitiba, Brazil; [2]Departamento de Bioquímica e Biologia Molecular, UFPR, Curitiba, Brazil; [3]Departamento de Anatomia, Instituto de Ciências Biomédicas-3, USP, São Paulo, Brazil

[*]**Author for correspondence:** Claudio Da Cunha, Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Departamento de Farmacologia, UFPR, C.P. 19.031, 81.531-980 Curitiba PR, Brazil. Tel: +55 41 3361-1717. Fax: +55 41 3266-2042. E-mail: dacunha@ufpr.br

Running title: The MPTP rat model of Parkinson's disease

Summary

The screening of drugs to treat Parkinson's disease (PD) has improved notably after the advent of the 6-hydroxydopamine (6-OHDA) rat model, which resembles the end stage of the disease. Injected into the medial forebrain bundle, this neurotoxin causes an almost total loss of mesencephalic dopamine neurons, upregulation of D2-like dopamine receptors in the striatum, and contralateral turning behaviour when unilaterally lesioned rats are challenged with dopamine receptor agonists. The purpose of the present study was to validate another rat model that resembles the early stage of PD characterized by partial loss of dopamine neurons. Male Wistar rats received an infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the substantia nigra, compact part (SNc). The partial loss of tyrosine hydroxylase-immunoreactive neurons mainly restricted to the SNc and the partial depletion of striatal dopamine levels in the dorsal striatum showed that this model was successful in mimicking the early stage of PD. These MPTP rats presented dose-dependent ipsilateral turning behaviour when challenged either with the dopamine receptor agonist, apomorphine, or with the indirect dopamine receptor agonist, amphetamine. This behaviour also qualitatively differed from that observed in 6-OHDA rats, which presented contralateral turns when challenged with apomorphine and ipsilateral turns when challenged with amphetamine. The present results validate the ipsilateral turning behaviour of MPTP rats as a simple and quantitative model with predictive value for the screening of drugs potentially effective to improve the motor impairments observed in the early stage of PD.

Keywords: Parkinson's disease; MPTP; 6-OHDA; rat; dopamine; turning behaviour; substantia nigra.

Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SNc, substantia nigra, compact part; MFB, medial forebrain bundle.

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 (see Olanow and Tatton, 1999). These drugs range from the dopamine precursor levodopa to competitive dopamine receptor agonists, including dopamine-degrading enzyme inhibitors (Ives *et al*., 2004; Poewe, 2004; Thobois, 2006). The antiparkinsonian effect of these drugs can be predicted by their property to induce a turning behaviour in rats with unilateral lesion of the substantia nigra, compact part (SNc) caused by intracerebral infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) (Ungerstedt & Arbuthnott, 1970; Schwarting & Huston, 1996b; Schober, 2004).

The predictive basis of this turning behaviour has been better studied by challenging the animals with the dopamine receptors agonist, apomorphine, and the indirect dopamine receptor agonist, amphetamine. Amphetamine is considered to be an "indirect agonist" because it induces the release of dopamine (Schwarting & Huston, 1996b). The almost total loss of nigral dopamine neurons usually results in supersensitivity of the postsynaptic dopamine receptors in the striatum (Ungerstedt, 1971; Thal *et al*., 1979). Therefore, when a unilaterally lesioned rat is challenged with apomorphine, the upregulated dopamine receptors in the ipsilateral striatum will be more strongly stimulated than the receptors of the contralateral side. This misbalance results in a turning behaviour toward the opposite side of the lesion, what is usually called contraversive turning behaviour (Schwarting & Huston, 1996a). On the other hand, challenging unilaterally 6-OHDA-lesioned rats with amphetamine induces turns toward the lesioned side, i.e., an ipsiversive turning behaviour (Ungerstedt & Arbuthnott, 1970). The direction of the turns to the side opposite to those induced by apomorphine is explained by the fact that amphetamine induces the release of dopamine only in the presynaptic terminals of the non-lesioned side. In the striatum of the lesioned side, most of the dopamine neuron terminals are lost after 6-OHDA lesion. Therefore, the postsynaptic dopamine receptors of the striatum contralateral to the lesion will be more strongly stimulated than those of the ipsilateral side, thus inducing ipsiversive behaviour.

Although the 6-OHDA rat model was developed based on the effect of amphetamine and apomorphine, it is effective in predicting even the antiparkinsonian effects of drugs acting through non-dopaminergic mechanisms (e.g., glutamatergic) (Moreli, 1997; Dekundy *et al*., 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 that stage, patients have lost most of the dopamine neurons in the SNc (Braak *et al*., 2003) and present increased postsynaptic dopamine receptor density and/or supersensitivity in the putamen (Seeman & 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 & Frucht, 2005). The unilateral infusion of 6-OHDA into the rat striatum has been used as a model of early-stage PD (Berger *et al*., 1991; Ichitani *et al*.,1994; Lee *et al*., 1996; Przedborski *et al*., 1995; Sauer & Oertel, 1994; Yuan *et al*., 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. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered after drug addicts were accidentally intoxicated with the 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 SNc (Chassain *et al.*, 2001). Since then, MPTP-treated monkeys have been successfully used as a model of PD. However, few studies have employed rats because these animals proved to be more resistant to the neurotoxic effect of MPTP (Giovanni *et al.*, 1994a,b; Smeyne & Jackson-Lewis, 2005). Harik *et al*. (1987) reported that the intranigral infusion of a high dose of MPTP into the rat SNc caused its partial lesion and depletion of striatal dopamine in a more selective way than 6-OHDA. More recently, we have shown that, when MPTP is infused bilaterally into the SNc, it causes the same memory impairments in the water maze test as observed in 6-OHDA-lesioned rats (Ferro *et al*., 2005). However, tyrosine hydroxylase (TH)-immunostained cell loss in the SNc, dopamine depletion in the striatum, and animal mortality were markedly lower in MPTP rats. This 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, 2002, 2003; Gevaerd *et al*., 2001a,b; Miyoshi *et al*., 2002; Bellissimo *et al*., 2004; Braga *et al*., 2005; Perry *et al*., 2005).

The aim of the present study was to validate the use of the rat MPTP model of the early stage of PD as a screening test for putative drugs to treat motor disabilities of PD. Since robust and reproducible results were obtained when unilaterally 6-OHDA-lesioned rats were treated with apomorphine or amphetamine, we compared these responses to those observed for unilaterally MPTP-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 behavioural experiments were conducted between 07:00 and 13:00 h. 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).

Surgeries

Different sites of infusion were chosen for MPTP (SNc) and 6-OHDA (MFB) based on previous knowledge showing that they produced the more robust and reproducible results regarding SNc lesion and turning behaviour (Schwarting & Huston, 1996b; Deumens *et al*., 2002). In our experience, the infusion of MPTP into the rat MFB causes fewer loss of dopamine neurons in the SNc or turning behaviour in rats challenged with apomorphine or

amphetamine (unpublished data). Previous findings also guided the choice of a higher dose of MPTP compared to 6-OHDA. Almost total lesion of the SNc 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 SNc dopamine neurons when infused directly into the SNc 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 SNc (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 anaesthetised 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 animals treated with MPTP 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 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 SNc 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 & 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 the rats' crumbled chow and water for the first 5 postoperative days. This procedure reduced body weight loss and, consequently, mortality.

Turning behaviour test

One week after surgery, the animals were challenged with a subcutaneous injection of apomorphine or an i.p. injection of amphetamine (see figure legends for detail of doses and number of animals per group). 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 and measured for 1 h (Ungerstedt & 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 behaviour was scored as described above. Data of animals that made fewer than 50 turns (ipsilateral for MPTP and contralateral 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 & Huston, 1996b).

TH immunohistochemistry

After the behavioural tests, the animals were killed by decapitation and their dorsal striata were 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). The antigen-antibody complex was localized with an ABC Elite kit. 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), an L-ECD-6A electrochemical detector (Shimadzu), 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.

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, methanol and perchloric acid (all from Merck, Darmstadt, Germany), penicillin G-procaine (Bristol-Myers Squibb, New York, NY, USA), magnesium sulfate, ascorbic acid and propylene glycol (all from Synth, São Paulo, Brazil), sodium thiopental (Abbott Laboratories, Abbott Park, IL, USA), and atropine sulfate, acetaldehyde, amphetamine, apomorphine, citric acid, dopamine, desipramine, DOPAC, EDTA, HVA, MPTP HCl, heptanesulfonic acid and 6-OHDA (all from Sigma Chemical Co., St. Louis, MO, USA). The monoclonal antibody against TH raised in mice was purchased from Incstar Corp. (Stillwater, MN, USA), and the ABC Elite kit to localize the antigen-antibody complex was purchased from Vector Laboratories (Burlingame, CA, USA). The C-18 reverse-phase columns were purchased from Phenomenex (Torrance, CA, USA).

Statistical analysis

All results are reported as the mean ± SEM. Differences among groups were analysed 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 analysed 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 behaviour when challenged with apomorphine or amphetamine (Figure 1). Both MPTP and 6-OHDA rats showed ipsiversive turning behaviour when challenged with amphetamine. However, apomorphine caused ipsiversive turning behaviour in MPTP rats and contraversive turning behaviour 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 ($F(4, 39) = 1.93$, $P = 0.12$).

The results in Figure 2 show that the ipsiversive turning behaviour 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).

As illustrated in Figure 3, the time courses of the effect of apomorphine on the turning behaviour of MPTP and 6-OHDA rats presented opposite directions 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 behaviour remained almost constant for up to 35 min after the challenge in MPTP rats and for at least 60 min in 6-OHDA rats.

The loss of TH-immunoreactive neurons in the rat midbrain induced by MPTP or 6-OHDA is illustrated in Figure 4. MPTP caused neuronal loss which was mainly restricted to the SNc, spreading only modestly to the neighbouring brain areas. On the other hand, 6-OHDA caused an almost complete loss of TH-immunoreactive neurons in the SNc, ventral tegmental area, and retrorubral field (data not shown). Nissl staining showed that in both cases the substantia nigra, reticulate part 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).

Discussion

The present results show that unilateral lesion of the rat SNc with MPTP causes different effects in rats 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 & Huston, 1996a; Truong *et al*., 2006). On the other hand, MPTP caused a partial loss of dopamine neurons and striatal dopamine, also in agreement with previous studies (Harik *et al*., 1987; Da Cunha *et al*., 2001; Gevaerd *et al*., 2001a; Ferro *et al*., 2005). The study of other behavioural and toxic effects of these two models was beyond the scope

of the present investigation and a detailed description can be found elsewhere (Schwarting & Huston, 1996b; Ferro *et al*., 2005).

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 & Huston, 1996a; Ghorayeb *et al*., 2002; Deumens *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). This resistance is usually considered to be a reason not to use MPTP to lesion rats. However, this feature can be useful to model the early 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, 2003; Gevaerd *et al*., 2001a,b; Miyoshi *et al*., 2002; Bellissimo *et al*., 2004; Braga *et al*., 2005, Ferro *et al*., 2005). The most useful characteristic of these bilaterally MPTP-lesioned rats for cognitive studies was the lack of gross motor alterations.

The present study showed that unilaterally MPTP-lesioned rats also present motor alterations in response to dopamine drug challenges, and that this effect is robust and dose-dependent. Recent studies suggest that the contraversive turning behaviour of 6-OHDA rats is more correlated with dyskinetic effects of the challenging drug than with antiparkinsonian effects per se (Lane *et al.*, 2006; Konitsiotis & Tsironis, 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). Indeed, the ipsiversive turning behaviour 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-lesioned 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, therefore, in ipsiversive turning behaviour. An amphetamine challenge causes the release of endogenous dopamine (Schwarting & 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 behaviour. The higher potency of dopamine and amphetamine to induce turning behaviour in 6-OHDA rats compared to MPTP animals is probably due to the upregulation of dopamine receptors observed in 6-OHDA (Ungerstedt, 1971; Thal *et al*., 1979) but not in MPTP rats (Perry *et al*., 2005).

Seen from this perspective, the ipsiversive turning behaviour of MPTP rats would be modelling a phenomenon that occurs when early-stage PD patients are treated with dopaminergic drugs. This is an important period in the life of these patients, 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). Moreover, some of these drugs can aggravate the time course of the disease (Bonuccelli *et al.*, 2002). In addition, a great effort has been made in the search for neuroprotective drugs to be used in the early stage of PD (Wu & Frucht, 2005). In this respect, it makes sense to base the screening for these drugs on a PD model in which part of the dopamine neurons are still alive. For these reasons, we believe that MPTP rats are a simple and promising model for the study of neural alterations and for the screening of putative drugs to treat the early stage of PD. The dose-effect turning behaviour of MPTP rats challenged with apomorphine, in a direction opposite to that observed in 6-OHDA rats, can be a useful quantitative method for the study of motor effects of direct dopamine receptor agonists.

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.

References

Bellissimo, M.I., Kouzmine, I., Ferro, M.M., De Oliveira, B.H., Canteras, N.S. & Da Cunha, C. (2004). Is the unilateral lesion of the substantia nigra pars compacta sufficient to induce working memory impairment in rats? *Neurobiol. Learn. Mem.*, **82**, 150-158.

Berger, K., Przedborski, S., & Cadet, J.L. (1991). Retrograde degeneration of nigrostriatal neurons induced by intrastriatal injection in rats. *Brain Res. Bull.,* **26,** 301–307.

Bonuccelli, V., Napolitano, A., Del Dotto, P. & Quattrone, A. (2002). Motor response to apomorphine in patients with Parkinson's disease with long-duration response to levodopa. *Clin. Neuropharmacol.*, **25**, 119-121.

Braak, H., Del Tredici, K., Rub, U., De Vos, R.A.I., Steur, E.N.H.J., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease *Neurobiol. Aging*, **24**, 197-211.

Braga, R., Kouzmine, I., Canteras, N.S. & Da Cunha, C. (2005). Lesion of the substantia nigra, pars compacta impairs delayed alternation in a Y-maze in rats. *Exp. Neurol*., **192**, 134-141.

Chassain, C., Eschalier, A. & Durif, F. (2001). Assessment of motor behavior using a video system and a clinical rating scale in parkinsonian monkeys lesioned by MPTP. *J. Neurosci. Meth.*, **111**, 9-16.

Chiueh, C., Markey, S.P., Burns, R.S., Johannessen, J.N., Pert, A., Kopin, I.J. (1984). Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine in the rat. *Eur. J. Pharmacol.,* **100**, 189-194.

Da Cunha, C., Angelucci, M.E.M., Canteras, S.C., Wonnacott, S. & Takahashi, R.N. (2002). The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson's disease memory disabilities. *Cell. Mol. Neurobiol.*, **22**, 227-237.

Da Cunha, C., Gevaerd, M.S., Vital, M.A.B.F., Miyoshi, E., Andreatini, R., Silveira, R., Takahashi, R.N. & Canteras, N.S. (2001). Memory disruption in rats with nigral lesions induced by MPTP: a model for early Parkinson's disease amnesia. *Behav. Brain Res.*, **124**, 9-18.

Da Cunha, C., Wietzikoski, S., Wietzikoski, E.C., Miyoshi, E., Ferro, M.M., Anselmo-Franci, J.A. & Canteras, N.S. (2003). Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system. *Neurobiol. Learn. Mem*., **9**, 236-242.

Dekundy, A., Pietraszek, M., Schaefer, D., Cenci, A.A. & Danysz, W. (2006). Effects of group I metabotropic glutamate receptors blockade in experimental models of Parkinson's disease. *Brain Res. Bull.*, **69**, 318-326.

Deumens, R., Blokland, A. & Prickaerts, J. (2002). Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Exp. Neurol.*, **175**, 303-317.

Ferro, M.M., Bellissimo, M.I., Anselmo-Franci, J.A., Angellucci, M.E.M., Canteras, N.S. & Da Cunha, C. (2005). Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early stage of Parkinson's disease: Histological, neurochemical, motor and memory alterations. *J. Neurosci. Meth.,* **148**, 78–87.

Gevaerd, M.S., Miyoshi, E., Silveira, R., Canteras, N.S., Takahashi, R.N. & Da Cunha, C. (2001a). L-DOPA restores striatal dopamine level but fails to reverse MPTP-induced memory deficits in rats. *Int. J. Neuropsychopharmacol.*, **4**, 361-370.

Gevaerd, M.S., Takahashi, R.N., Silveira, R. & Da Cunha, C. (2001b). Caffeine reverses the memory disruption induced by intra-nigral MPTP injection in rats. *Brain Res. Bull.*, **55**, 101-106.

Ghorayeb, I., Fernagut, P.O., Hervier, L., Labattu, B., Bioulac, B. & Tison, F. (2002). A 'Single toxin-double lesion' rat model of striatonigral degeneration by intrastriatal 1-methyl-4-phenylpyridinium ion injection: a motor behavioural analysis. *Neuroscience*, **115**, 533-546.

Giovanni, A., Sieber, B.A., Heikkila, R.E. & Sonsalla, P.K. (1994a). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine. Part 1: Systemic administration. *J. Pharmacol. Exp. Ther.*, **270**, 1000-1007.

Giovanni, A., Sonsalla, P.K. & Heikkila, R.E. (1994b). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine. Part 2: Central administration of 1-methyl-4phenylpyridinium. *J. Pharmacol. Exp. Ther*., **270**, 1008-1014.

Harik, S.I., Schmidley, J.W., Iacofano, L.A., Blue, P., Arora, P.K. & Sayre, L.M. (1987). On the mechanisms underlying 1-methyl-4phenyl-1,2,3,6-tetrahydropiridine neurotoxicity: the effect of perinigral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine, its metabolite and their analogs in rat. *J. Pharmacol. Exp. Ther*., **241**, 669–676.

Ichitani, Y., Okamura, H., Ankara, D., Nagatsu, I., & Ibata, Y. (1994). Biochemical and immunocytochemical changes induced by intrastriatal 6-OHDA injection in the rat nigrostriatal dopamine neuron system: evidence for cell death in the substantia nigra. *Exp. Neurol.*, **130**, 269–278.

Ives, N.J., Stowe, R.L. & Marro, J. (2004). Monoamine oxidase type B inhibitors in early Parkinson's disease: meta-analysis of 17 randomised trials involving 3525 patients. *Brit. Med. J.*, **329**, 593-596B.

Kalaria, R.N., Mitchell, M.J. & Harik, S.I. (1987). Correlation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity with blood-brain barrier monoamine-oxidase activity. *Proc. Natl. Acad. Sci. USA*., **84**, 3521–3525.

Konitsiotis, S. & Tsironis, C. (2006). Levodopa-induced dyskinesia and rotational behavior in hemiparkinsonian rats: Independent features or components of the same phenomenon? *Behav. Brain Res.*, **170**, 337-341.

Lane, E.L., Cheetham, S.C. & Jenner, P. (2006). Does contraversive circling in the 6-OHDA-lesioned rat indicate an ability to induce motor complications as well as therapeutic effects in Parkinson's disease? *Exp. Neurol.*, **197**, 284-290.

Langston, J.W., Ballard, P., Tetrud, J.W. & Irwin, I. (1983). Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science*, **219**, 979-980.

Lees, A. (2005) Alternatives to levodopa in the initial treatment of early Parkinson's disease. *Drugs Aging*, **22**, 731-740.

Lee, C.S., Sauer, H., & Björklund, A. (1996) Dopaminergic neuronal degeneration and motor impairments following axon terminal lesion by intrastriatal 6-hydroxydopamine in the rat. *Neuroscience*, **72,** 641–653.

Miyoshi, E., Wietzikoski, S., Camplessei, M., Silveira, R., Takahashi, R.N. & Da Cunha, C. (2002). Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. *Brain Res. Bull.*, **58**, 41-47.

Morelli, M.S.(1997) Dopamine/glutamate interaction as studied by combining turning behaviour and c-fos expression. *Neurosci. Biobehav. Rev*. **21**, 505-509.

Olanow, C.W., & Tatton, W.G. (1999) Etiology and pathogenesis of Parkinson's disease. *Ann. Rev. Neurosci.*, **22**,123-144.

Paxinos, G., & Watson, C. (2005). The rat brain in stereotaxic coordinates (5th ed.). San Diego, CA: Academic Press.

Perry, J.C., Hipólide, D.C., Tufik, S., Martins, R.D., Da Cunha, C., Andreatini, R. & Vital, M.A.B.F. (2005). Intra-nigral MPTP lesion in rats: behavioral and autoradiography studies. *Exp. Neurol.*, **195**, 322-329.

Poewe, W. (2004). The role of COMT inhibition in the treatment of Parkinson's disease *Neurol*., **62**, S31-S38, Suppl. 1.

Przedborski, S., Levivier, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., & Donaldson, D. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. *Neuroscience,* **67**, 631–647.

Riachi, N.J., Harik, S.I., Kalaria, R.N., & Sayre, L.M. (1988). On the mechanisms underlying 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity: II. Susceptibility among mammalian species correlates with the toxin's metabolic patterns in brain microvessels and liver, *J. Pharmacol. Exp. Therap.*, **244**, 443– 448.

Sauer, H., & Oertel, W.H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience*, **59**, 401–415.

Schober, A (2004). Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell Tissue Res.*, **318**, 215-224.

Schwarting, R.K. & Huston, J.P. (1996a). Unilateral 6-hydroxydopamine lesions of the meso-striatal dopamine neurons and their physiological sequelae. *Prog. Neurobiol.*, **49**, 215-266.

Schwarting, R.K. & Huston, J.P. (1996b). The unilateral 6-hydroxydopamine lesion model in behavioral brain research: analysis of functional deficits, recovery and treatments. *Prog. Neurobiol.*, **50**, 275-331.

Seeman, P. &. Niznik, H. B.(1990). Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *FASEB J.*, **4**, 2737–2744.

Smeyne, R.J. & Jackson-Lewis, V. (2005). The MPTP model of Parkinson's disease. *Mol. Brain Res*., **134**, 57– 66.

Thal, L., Mishra, R.K., Gardner, E.L., Horowitz, S.G., Varmuza, S., & Makman, M.H. (1979). Dopamine antagonist binding increases in 2 behaviorally distinct striatal denervation syndromes. *Brain Res.*, **170**, 381-386.

Thobois, S. (2006). Proposed dose equivalence for rapid switch between dopamine receptor agonists in Parkinson's disease: A review of the literature. *Clin. Therap*., **28**, 1-12.

Truong, L., Allbutt, H., Kassiou. M., Henderson, J.M. (2006). Developing a preclinical model of Parkinson's disease: A study of behaviour in rats with graded 6-OHDA lesions. *Behav. Brain Res.*, **169**, 1-9.

Ungerstedt, U. & Arbuthnott, G.W. (1970). Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.*, **24**, 485-493.

Ungerstedt, U. (1968). 6-Hydroxydopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmacol.*, **5**, 107-110.

Ungerstedt, U. (1971). Postsynaptic supersensitivity after 6-hysroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta. Physiol. Scand*., **367**, 69-93.

Wu, S. S. & Frucht, S.J. (2005). Treatment of Parkinson's disease: what's on the horizon? *CNS Drugs*, **19**, 723-743.

Yuan, H., Sarre, S., Ebinger, G., & Michotte, Y. (2005). Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson’s disease. *J. Neurosci. Meth.*, **144**, 35-45.

Figure Legends:

Figure 1 Turning behaviour 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).

Figure 2 Turning behaviour of unilaterally MPTP-lesioned rats challenged with 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).

Figure 3 Time course of the apomorphine-challenged turning behaviour of unilaterally MPTP- or 6-OHDA-lesioned rats. 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.

Figure 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; SNc = substantia nigra, compact part; SNr = substantia nigra, reticulate part; VTA = ventral tegmental area. Scale bars: 500 µm.

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

	STRIATAL DAPAMINE (ng/g wet tissue)	
	LEFT	RIGHT
Sham	5161.29 ± 369.06	5248.32 ± 333.32
MPTP-Left	1698.26 ± 296.15 *#	5005.60 ± 1392.15
MPTP-Right	4984.20 ± 1003.53	2558.74 ± 617.19 *#
MPTP-Bilateral	1016.26 ± 569.77 #	995.74 ± 502.37 #
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 rats (Newman Keuls test after one-way ANOVA).

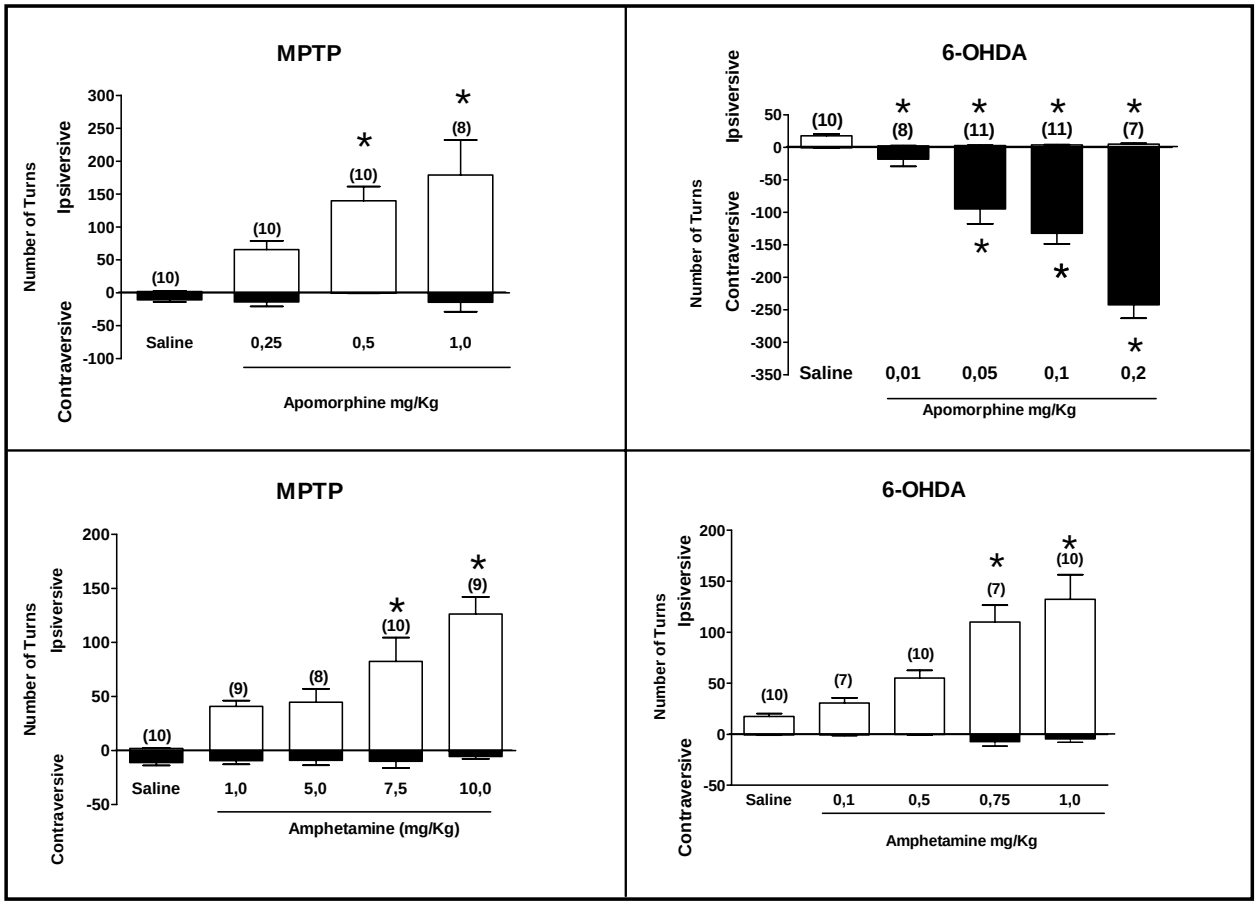

Figure 1 Turning behavior of MPTP and 6-OHDA unilaterally lesioned rats challenged with apomorphine or amphetamine. Data expresses the number of ipsiversive (positive scale) and contraversive turns (negative scale) counted during the first 30 min after the drug challenge. The number animals per group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

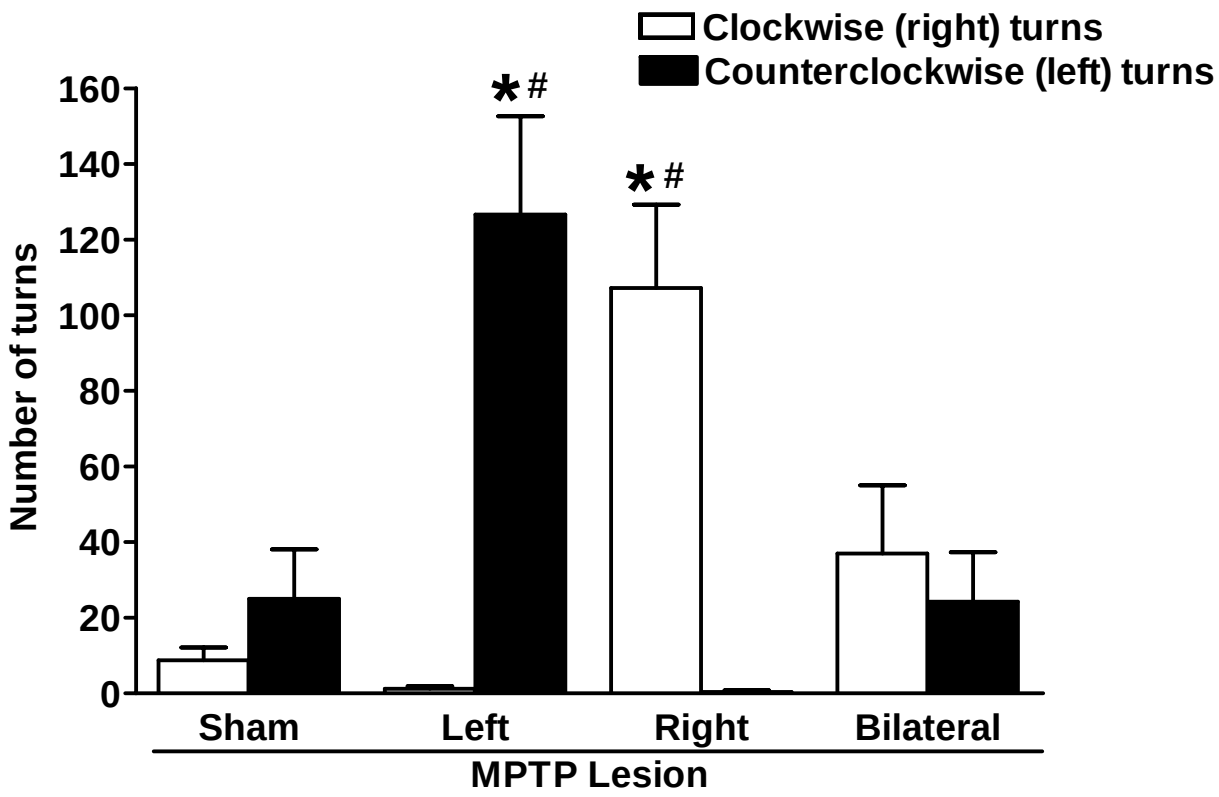

Figure 2 Turning behavior of unilaterally lesioned MPTP rats challenged with apomorphine. Data expresses the number of clockwise and counterclockwise turns counted during the first 30 min after the drug challenge. The number animals per group were the following: 12 sham, 10 right, 10 left, 8 bilateral. * P < 0.05 compared to sham rats; # p < 0.05 compared to rats lesioned in the contralateral side; Newman Keuls after one-way ANOVA.

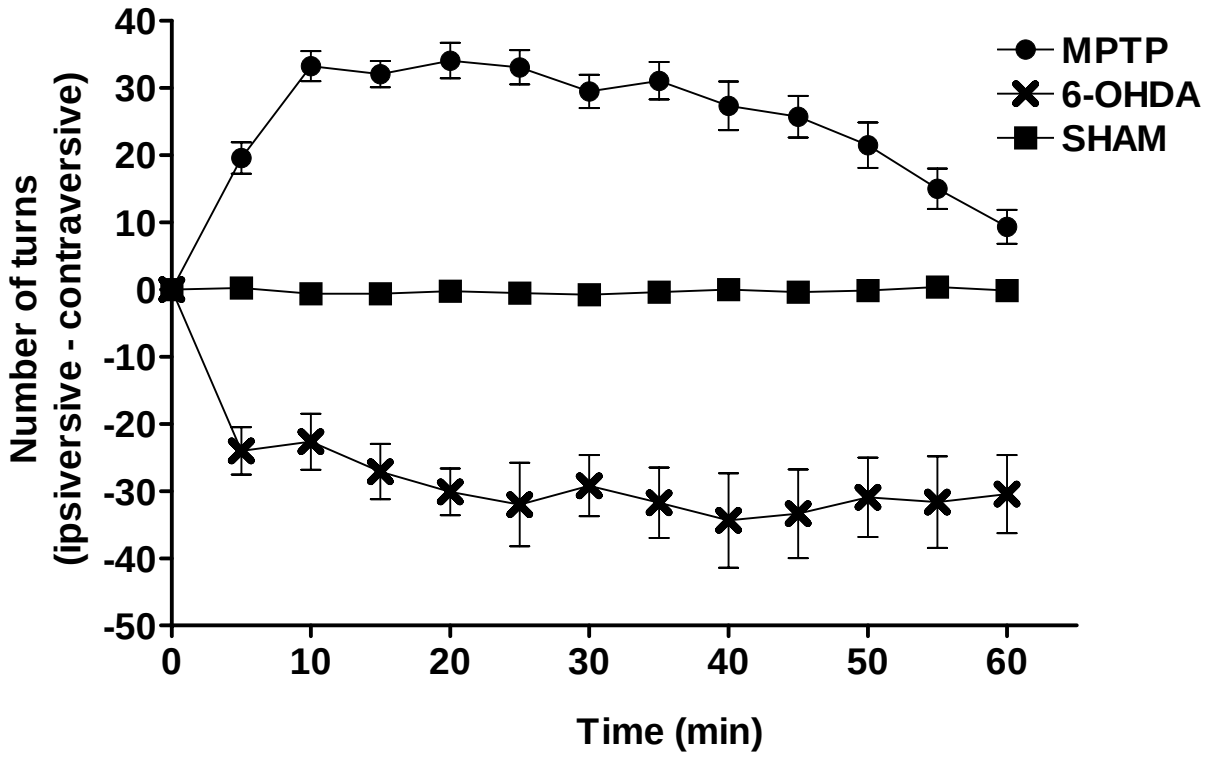

Figure 3 Time course of the apomorphine challenged turning behavior of unilaterally MPTP or 6-OHDA lesioned rats. Data expresses the number of ipsiversive - contraversive turns counted in intervals of 5 min. The number of animals per group were the following: 8 sham, 11 MPTP, 12 6-OHDA. A two-way ANOVA followed by the Newman Keuls test found significant differences ($P < 0.05$) among the scores of the three groups at all time intervals.

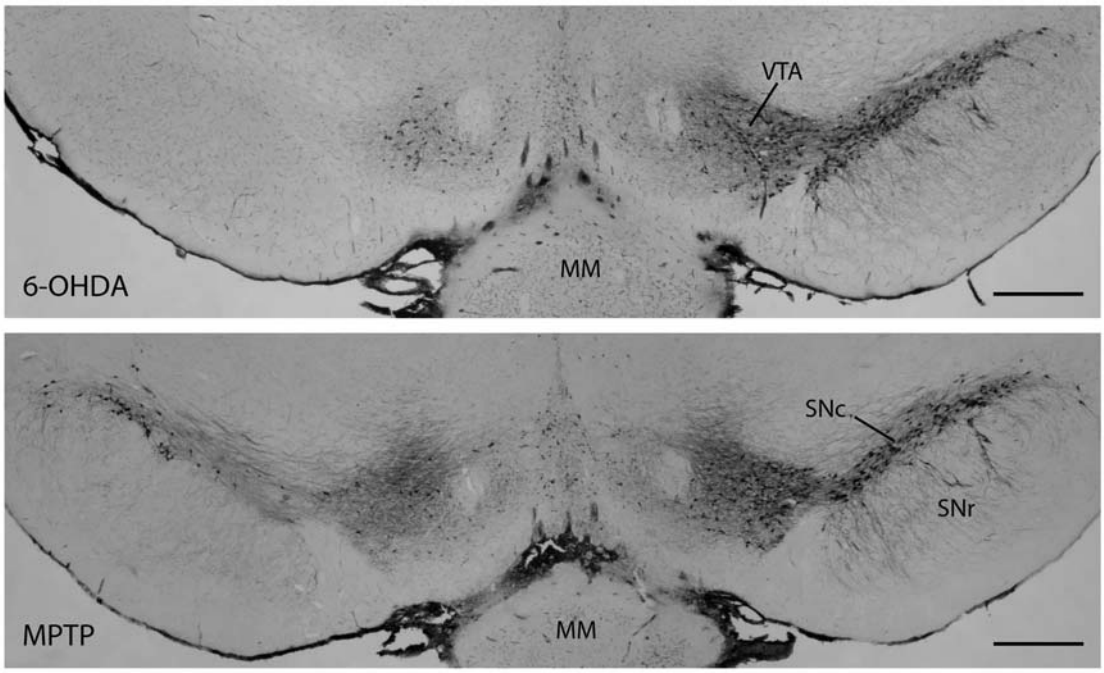

Figure 4 Representative brightfield photomicrographs of tyrosine hydroxylase-immunostained section illustrating the appearance of the mesencephalum of rats that received a periningral infusion of MPTP or a intra-medial forebrain bundle infusion of 6-OHDA. SNc = substantia nigra, compact part; SNr = substantia nigra, reticulate part; VTA = ventral tegmental area.

EXPERIMENTO 2

O TAMANHO DA LESÃO UNILATERAL NA SUBSTÂNCIA NEGRA COMPACTA DETERMINA O SENTIDO DA ROTAÇÃO EM RATOS DESAFIADOS COM APOMORFINA

Trabalho submetido para publicação na revista The Journal of Neuroscience, 2006.

Section: Neurobiology of Disease
Senior Editor: Dr. Karl Herrup

Title: The size of the unilateral lesion of the substantia nigra pars compacta determines the direction of turns in rats challenged with apomorphine

Abbreviated Title: Turning behavior of SNc lesioned rats

Claudio Da Cunha[1], Evellyn C. Wietzikoski[1], Marcelo M. Ferro[2], Gláucia Regina Martinez[2], Jeff C. Pedrozo Jr[1], Maria A. B. F. Vital [1], Débora Hipolide[3], Sergio Tufik[3], and Newton S. Canteras[4]

[1]Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Departamento de Farmacologia, UFPR, Curitiba, Brazil; [2]Departamento de Bioquímica e Biologia Molecular, UFPR, Curitiba, Brazil; [3]Departamento de Psicobiologia, UNIFESP, São Paulo, Brazil; [4]Departamento de Anatomia, Instituto de Ciências Biomédicas-3, USP, São Paulo, Brazil.

Correspondence should be addressed to Claudio Da Cunha, Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Departamento de Farmacologia, UFPR, C.P. 19.031, 81.531-980 Curitiba PR, Brazil. Tel: +55 41 361-1717. Fax: +55 41 266-2042. E-mail: dacunha@ufpr.br
5 Figures
28 Pages

Key Words: substantia nigra pars compacta; turning behavior; dopamine; autoradiography; dopamine receptor; Parkinson's disease.

Acknowledgements: This research was supported in part by CNPq, CAPES, FAPESP, Institutos do Milênio, and Fundação Araucária. The skillful assistance of Vinicius A. Lima, Scheila M. G. Takayasu, and Michele L. Gregório with some of the HPLC assays and behavioural experiments are acknowledged.

ABSTRACT

Turning behavior was observed in adult male Wistar rats with unilateral lesion of the substantia nigra pars compacta (SNc) challenged with the dopamine receptor agonist, apomorphine. A lesion induced by 6-hydroxidopamine (6-OHDA) caused contraversive turns when infused into the medial forebrain bundle (MFB) or into the medial or central parts of the SNc, and few ipsiversive turns when infused into the lateral part of the SNc. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) always induced ipsiversive turns when infused into any of these sites, except the medial part of the SNc. In all cases the direction of the turns could be predicted by the magnitude of tyrosine-hydroxylase immunorreactive neuronal loss in the mesecephalon and by dopamine depletion in the striatum: very small lesions did not cause turns, small lesions caused ipsiversive turns, and large (almost total) caused contraversive turns. Supersensitivity of D2-like dopamine receptors, evaluated by [3H]raclopride binding, observed only in the striatum of rats with an almost total loss of dopamine/neurons, seems to be the critical to reverse the direction of the turns from ipsiversive to contraversive. These results are coherent with the hypothesis that the SNc unilaterally lesioned animals rotates toward the side with the weaker activation of dopamine receptors. This activation is weaker in the lesioned side of animals with small lesions of the SNc (less endogenous dopamine), but stronger in the animals with an almost total lesion due to the supersensitivity of the dopaminergic receptors.

Introduction

Turning behavior of rats with unilateral lesion of the substantia nigra pars compacta (SNc) induced by intracerebral infusion of 6-hydroxydopamine (6-OHDA) challenged with dopaminergic drugs was proposed as a model of Parkinson's disease (PD) by Ungerstedt in 1968. Since then, it has been one of the most popular models to study drugs potentially useful to treat this disease (Gerlach and Riederer, 1996; Schwarting and Huston, 1996b; Yuan et al., 2005). More than 7000 papers have been published using this model. However, there is no consensual explanation for the direction of the turns made by these rats.

Most studies showed rats with unilateral lesions induced by 6-OHDA presenting contraversive turning behavior (in the opposite direction of the lesioned side) when challenged with apomorphine. However, ipsiversive or few turns in both directions have also been reported in animals with unilateral lesions induced by 6-OHDA (Ungerstedt, 1971; Costall et al., 1976; Gardner et al., 1976; Thal et al., 1979; Redgrave and Mitchell, 1982; Annet et al., 2006; Olds et al., 2006), and other neurotoxins (e.g. rotenone, MPP^+) (Jasso-López and Tapia, 1995; Kondo et al., 2004; Sindhu et al., 2006). Turning behavior seems to be caused by a dysbalance between the right- and left-side drives to initiate movements due to striatal outputs which is modulated by the nigrostriatal pathway (see Nicola et al., 2000).

The site of infusion of 6-OHDA has been said to be critical to explain the direction of the turns (Redgrave and Mitchell, 1982; Schwarting and Huston, 1996a; Sindhu et al., 2006). The medial forebrain bundle (MFB) is pointed out as the infusion site that causes more contraversive turns after an apomorphine challenge. However, ipsiversive behavior is observed when these animals are challenged with amphetamine (Ungerstedt, 1971). Supersensitivity of dopamine receptors in the striatum was also suggested as an important factor to induce contraversive turns (Thal et al., 1979). Some authors had suggested the involvement of other neurotransmitter systems besides the dopamine (Hirschhorn et al., 1983).

In the present study, more than describing the critical factors determining the occurrence and the direction of the turning behavior in unilaterally SNc-

lesioned rats, we tried to find a rational explanation for this phenomenon. The hypotheses that the site of the lesion in the SNc, its size or magnitude, and its relation with supersensitity of dopamine receptors in the striatum were investigated. Non-dopaminergic contributions to the phenomena were not discarded, but a simpler explanation of the core of the phenomenon with exclusively dopaminergic components is offered.

Materials and Methods

Subjects. 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 07:00 a.m.) with food and water available ad libitum. All experimental procedures were in compliance with the National Institute of Health and the Brazilian Society for Neuroscience and Behavior guidelines and were approved by the Institutional Animal Care and Use Committee of the Federal University of Parana State.

Surgeries. The animals received atropine sulfate (0.4 mg/kg, i.p.) to suppress salivation, penicillin G-procaine (20,000 U in 0.1 ml, i.m.) to avoid infection, and were anaesthetised with 3 ml/kg equitesin (1% sodium thiopental, 4.25 % chloral hydrate, 2.13 % magnesium sulphate, 42.8% propylene glycol, and 3.7 % ethanol in water). The animals lesioned with MPTP received 3 i.p. injections of 120 mg/kg acetaldehyde 10 min before, at the beginning, and just after surgery, to increase the neurotoxin effectiveness. The animals lesioned with 6-OHDA received 25 mg/kg desipramine (i.p.) 10 min before surgery to avoid loss of noradrenergic neurons. MPTP (100 μg/μl in saline) or 6-OHDA (8 μg/μl in saline supplemented with 0.2% ascorbic acid) was infused through a 30-gauge stainless needle at 0.25 μl/min. The needle was maintained in place for more 2 min to avoid reflux. Fig. 1 illustrates the different sites where MPTP or 6-OHDA were infused and representative slices of the midbrain showing the loss of TH-immunostained neurons. The volumes and coordinates in which the neurotoxins were infused were the following: Medial part of the SNc: 1 μl, anteroposterior (AP), -5.9 mm from bregma; mediolateral (ML), ±1.2 mm from midline;

dorsoventral (DV), -8.6 mm from the skull; nose bar, - 3.3 mm from interaural line. Central part of the SNc: 1 μl, AP, -5.0 mm ML, ±2.1 mm; DV, -7.7 mm. Lateral part of the SNc: 1 μl, AP, -6.1 mm ML, ±2.6 mm; DV, -7.3 mm. Animals that received MPTP into the 3 sites of the SNc: same coordinates described above, 1 μl into the medial, 2 μl into the central, and 2 μl into the lateral part of the SNc. MFB: 2 μl: AP, -1.9 mm ML, +1.9 mm; DV, -7.2 mm. These stereotaxic coordinates were adapted from the Paxinos and Watson Atlas (2005). Sham-operated animals were submitted to the same surgical procedure, including the hole in the skull, but the infusion needle was not lowered into the brain. Other animals were submitted to the same procedure and saline was infused instead of the neurotoxins. After surgery, the animals were allowed to recover from anesthesia in a temperature-controlled chamber and then returned to their home cage. The animals were fed a pasty diet consisted of a mixture of crumbled chow and water during the first 5 postoperative days. This procedure reduced body weight loss and mortality.

Turning behavior test. One week after surgery the animals were challenged with a 1 mg/kg s.c. injection of apomorphine (see the number of animals per group in the Figure legends). Immediately after the injection, the rats were individually placed in a 28 cm diameter, 25 cm high, round plastic container and the number of 360º turns toward the side of the lesion (ipsiversive) and towards the opposite side (contraversive) were video-recorded and measured for 1 h (Ungerstedt and Arbuthnott, 1970).

Tyrosine hydroxylase immunohistochemistry. After the behavioral test, the animals were killed by decapitation and their dorsal striata were removed for the determination of dopamine concentration (see below). The posterior part of the rat brains were 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 for tyrosine hydroxylase (TH) with a monoclonal antibody against TH (1:5000 dilution). The antigen-antibody complex was localized with an ABC Elite kit.

Slides were then dehydrated and coverslipped with DPX. The number of TH-immunoreactive (TH-ir) neurons was determined using the 10X objective of a Axiophot Stereo HB050 (Zeiss, Germany) microscope equipped with a camera lucida by using the softwared UTHSCSA Image Tool version 2.0 (University of Texas, San Antonio, USA) for counting these neurons on both sides of the ATV, SNc, and retro-rubral nucleus in 8 series of coronal sections based on the Paxinos and Watson atlas (2005): -4,8 mm, -5,2 mm, -5,6 mm, -5,8 mm, -6,04 mm, -6,3 mm, -6,72 mm, -7,04 mm from bregma. The borders defining the ATV, SNc, and retro-rubral nucleus were determined from adjacent Nissl-stained sections that served as a reference for cytoarchitectural purposes.

Determination of dopamine and metabolites by HPLC-ED. The endogenous levels of dopamine and its non-conjugated metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by reverse-phase HPLC with electrochemical detection (ED). The system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 x 4.6 mm i.d., 4 μm particle size), a L-ECD-6A electrochemical detector (Shimadzu), a 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 an ultrasonic processor (Sonics) cell disrupter in 0.1 M perchloric acid. After centrifugation at 15,000 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, 10 ml tetrahydrofuran, pH 3.0. The peak areas of the external standards were used to quantify the sample peaks.

Autoradiographic binding assays. Seven days after surgery, rats from the salina-3 (n=8), salina-MFB (n=8), MPTP-3 lesion (n=8) and 6OHDA-MFB (n=8) were sacrificed. The brains were removed quickly, frozen over dry ice and stored at -80°C. Twenty-micron coronal sections were cut on a Leica cryostat at -20°C and mounted on washed gelatin-coated slides at approximately 0.3

mm intervals through the anterior–posterior extent of the brain. Slides were stored at -80° C until assayed. [3H]SCH 23390 binding to D1 receptor was performed as described previously (Nobrega et al., 1996). [^{3}H]raclopride binding to D2 receptor were carried out with following previously described procedures (Sasaki et al., 1997). Briefly, slices were brought to room temperature and then preincubated in 50 mM Tris–HCl; 120 mM NaCl; 4 mM MgCl2; 1 mM EDTA; 1.5 mM CaCl2 2H2O buffer (pH 7.4) for either 30 min (D1) or 15 min (D2). Sections were then incubated either in fresh buffer containing 2 nM [3H]SCH 23390 (85.0 Ci/mmol, PerkinElmer Life Science, Boston, USA) for 90 min at room temperature (D1), or in buffer containing 2 M [^{3}H]raclopride (60.1 Ci/mmol, PerkinElmer Life Science, Boston, MA) for 120 min at room temperature (D2). Additional sets of slices were incubated either in the presence of 2 μM butaclamol (D1), or 10 μM sulpiride (D2), for determination of non-specific binding. Sections were then washed in buffer and allowed to dry at room temperature. Slices were exposed to Kodak BioMax MR films in tungsten cassettes together with calibrated standards for 4 weeks. Films were developed and densitometric analyses performed using an M2 MCID system (Imaging Research, St. Catharine's, Ontario, Canada). Anatomical regions were defined according to Paxinos and Watson (2005). Multiple readings were obtained by an investigator who was unaware of group membership and averaged over serial sections for each brain. Density values for striatum region were first averaged within each section, then across sections within each brain, and then across subjects within each of the group.

Statistics. All results are presented as the mean ± SEM. Differences among groups were analyzed by one-way ANOVA, followed by the Newman Keuls test. Data of the time course of apomorphine induced turns were scored as number of ipsiversive – contraversive turns and analyzed by two-way ANOVA with repeated measures, followed by the Newman Keuls test. Differences were considered to be statistically significant when $p < 0.05$.

Results

Effects of the unilateral infusion of 6-OHDA or MPTP on different sites of the nigrostriatal pathway on the turning behavior of rats

The effects of these treatments on apomorphine-induced turning behavior are shown in Fig. 2. The infusion of saline *per se* into the nigrostriatal pathway induced ipsilateral turns compared to the sham group ($F(6,65) = 37.99$, $p < 0.001$, one way ANOVA; $p < 0.05$ Newman-Keuls post-hoc test, see Fig. 2A). The infusion of MPTP into the central, lateral, or central+medial+lateral sites of the SNc, but not into the medial part of the SNc or into the MFB induced more ipsiversive turns compared to saline group ($p < 0.05$ Newman-Keuls test). Equivalent results were found by the comparison of the time course curves of the apomorphine effect. Two-way ANOVA showed significant effects for site of infusion ($F(6,65) = 37.99$, $p < 0.001$), time ($F(11,715) = 17.43$, $p < 0.001$), and interaction site X time ($F(66,715) = 3.80$, $p < 0.001$) (see Fig. 2B for details).

As can be seen in Fig. 2A', rats lesioned with 6-OHDA infused into the MFB or into the medial part of the SNc presented contraversive turns significantly different from saline groups ($F(5,52) = 14.65$, $p < 0.001$, one way ANOVA; $p < 0.05$ Newman-Keuls test). Contraversive turning behavior was also observed in most, but not all the animals that received 6-OHDA into the central part of the SNc. However, no significant differences were observed comparing group or the one that received 6-OHDA into the lateral part of the SNc with sham or saline groups ($p > 0.2$, Newman-Keuls test). Moreover, infusion of 6-OHDA into the lateral part of the SNc caused ipsiversive turns significantly different from sham ($F(5,52) = 3.43$, $p < 0.01$, one way ANOVA; $p < 0.05$ Newman-Keuls test), but not from saline group. Equivalent results were found by the comparison of the time course curves of the apomorphine effect. Two-way ANOVA showed significant effects for site of infusion $F(5,52) = 13.44$, $p < 0.001$, but not for time $F(11,572) = 0.46$. A significant interaction site X time was found ($F(55,572) = 1.43$, $p < 0.05$) (see Fig. 2B' for details).

Effect of the size of the SNc lesion on turning behavior

The results presented above showed that MPTP-induced lesions caused ipsiversive or no turning behavior. 6-OHDA, on the other hand, caused

contraversive, ipsiversive or no turning behavior. The predominant effect depended on the site of injection, but the administration of 6-OHDA into different sites of the SNc caused turns in opposite directions. Therefore, it is possible that the size of the lesion, roughly determined by the neurotoxin type and its site of injection is the critical factor to induce ipsiversive or contraversive behavior. We tested this hypothesis by grouping 6-OHDA or MPTP rats by their turning behavior (ipsiversive, contraversive or none), irrespective to the site that this neurotoxin was injected in their SNc.

The following conclusions can be taken based on the number of remaining midbrain TH-immunostained neurons, striatal dopamine and its metabolites after these treatments, as shown in Fig. 3: 1) very small lesions of the SNc (blue color bars) caused no turning behavior; 2) small lesions (green bars) caused ipsiversive turns; 3) large lesions (red bars) caused contraversive turns. It occurred irrespective of the site in which the neurotoxins were infused.

One-way ANOVA followed by the Newman Keuls test led to this conclusion from data of loss of TH-immunostained neurons as illustrated in the Fig. 3A ($F(1,6) = 18.93$, $p < 0.01$,) and 3A' ($F(3,19) = 11.89$, $p < 0.001$. The same conclusion can be achieved from data of the loss of dopamine and its metabolites in the striatum: Dopamine after MPTP treatment: Fig. 3B: $F(2,23) = 24.00$, $p < 0.001$; Dopamine after 6-OHDA treatment: Fig. 3B': $F(3,23) = 52.45$, $p < 0.001$; DOPAC after MPTP treatment: Fig. 3C: $F(2,23) = 5.10$, $p < 0.05$; DOPAC after 6-OHDA treatment: Fig. 3C': $F(3,23) = 27.92$, $p < 0.001$. Significant differences among the groups were also observed in HVA after 6-OHDA treatment: Fig. 3D': $F(3,23) = 40.64$, $p < 0.001$; but not after MPTP treatment: Fig. 3D: $F(2,23) = 1.16$, $p = 0.33$.

Effect of the size of the SNc lesion on binding to D1 and D2 dopamine receptors in the striatum

As can be seen in the results described above, even the animals that received the MPTP into 3 sites in the SNc presented a medium size lesion of the SNc and ipsiversive turns. On the other side, all animals that received 6-OHDA into the MFB presented a large size lesion of the SNc and contraversive turning behavior. As can be seen in Fig. 4, large-size nigral lesions induced by infusion of 6-OHDA into the MFB caused an increase in the binding to D2

receptors labeled with [^{3}H]raclopride in the ipsilateral striatum. On the other hand, the lower lesions induced by MPTP reduced the binding to D1 receptors labeled with [^{3}H]SCH-23390 in the ipsilateral striatum, as shown by one-way ANOVA followed by the Newman Keuls test: Fig. 4A', [^{3}H]raclopride binding in 6-OHDA treated rats ($F(3,144) = 3.24$, $p < 0.05$); Fig. 4B, [^{3}H]SCH-23390 binding in MPTP treated rats ($F(3,140) = 2.99$, $p < 0.05$). No significant effect was found for [^{3}H]raclopride binding in MPTP treated animals (Fig. 4A, $F(3,153) = 1.58$, $p = 0.19$) and for [^{3}H]SCH-23390 binding in 6-OHDA animals (Fig. 4B', $F(3,166 = 1.90$, $p = 0.13$).

Discussion

The results of the present study do not suggest that the site of the lesion within the SNc is the critical factor to determine the direction of the turns induced by apomorphine in rats. It is true that the infusion of a specific neurotoxin into some specific sites along the nigrostriatal pathway caused a predominant pattern of turning behavior. However, the direction of the turns cannot be predicted only by the site in which the neurotoxin is infused. It is clear by the present findings that the infusion of 6-OHDA, but not MPTP, into the MFB and in the more medial part of the SNc caused contraversive turns. MPTP always caused ipsiversive or no turning behavior. 6-OHDA caused predominantly contraversive turns, except for infusion in the most lateral part of the SNc, which caused predominantly ipsiversive turns. Besides, even when receiving infusion of 6-OHDA into the medial or central parts of the SNc, some 6-OHDA rats presented ipsiversive behavior. Some previous studies had reported this variable turning behavior for 6-OHDA rats (Costall et al., 1976; Thal et al., 1979; Redgrave and Mitchell, 1982; Hirschhorn et al., 1983; Schwarting and Huston, 1996b; Olds et al., 2006). In order to work with a more homogeneous sample, many authors excluded the 6-OHDA rats that were not contraversive (Schwarting and Huston, 1996b; Takeda et al., 2005; Lane et al., 2006).

The apparently chaotic pattern of results described above can make sense if explained, not as a result of the site of the lesion, but rather as function

of the loss of midbrain dopaminergic neurons and striatal depletion of dopamine, irrespective to the lesion site. Before discussing the evidences raised in favor of this second hypothesis, let's see if the pattern of lesion can give a clue about the neurotoxic mechanisms of MPTP and 6-OHDA. When 6-OHDA was infused in a site with a high density of dopaminergic fibers, it caused a large lesion of the neurons from which they originate. It was the case of the infusion of 6-OHDA into the MFB. In this case nearly all SNc dopaminergic neurons were lost (see Fig. 1 and also Yuan et al., 2005; Marin et al., 2006; Thuong et al., 2006). The fibers that arise from neurons of the medial and central parts of the SNc leave it by passing though the most medial and ventral parts of the SNc, from which they enter into the MFB (Falon and Loughlin, 1985; Mori et al., 1999). Therefore, the more medial and ventral the site of 6-OHDA infusion, the more the neurotoxin will be in contact with dopaminergic fibers. The present and previous studies have found that more medial and ventral infusions of 6-OHDA caused the greater and more robust contraversive turning behavior in rats (Costall et al., 1976; Redgrave and Mitchell, 1982; Schwarting and Huston, 1996b; Olds et al., 2006). These findings suggest that 6-OHDA is uptaken mainly by the dopamine uptake transporter (DAT) found in dopaminergic fibers.

On the other hand, MPTP did not cause a large loss of midbrain dopaminergic neurons when it was infused in sites rich in dopaminergic fibers, like the MFB and the ventro-medial part of the SNc. As far as we know, there are no sstudies suggesting that the toxic metabolite of MPTP, MPP^+, is uptaken preferentially by the dopaminergic neuron bodies/dendrites in relation to axons. Nevertheless it is what we can suggest, based on the present results. However, rats are more resistant to the neurotoxic effects of MPTP compared to primates (Giovanni et al., 1994; Blum et al., 2001). Therefore, even infusing a highly concentrated solution of MPTP simultaneously into 3 sites throughout the SNc, the loss of dopaminergic neurons was not as great as that caused by the infusion of 6-OHDA into the MFB. This treatment mimicked in area, but not in magnitude, the spread loss of midbrain dopaminergic neurons that resulted from the 6-OHDA infusion into the MFB. The ipsiversive turning behavior of the 3 sites MPTP rats, contrasting with the contraversive turning behavior of the MFB 6-OHDA rats, strongly suggest that it was not the site or area, but the

amount of dopaminergic neuron loss and the consequent striatal dopamine depletion that determines the direction of the turns.

This hypothesis was further tested by grouping animals that received 6-OHDA or MPTP into different sites by their turning behavior. This grouping criterion suggested that there are two critical thresholds determining the direction of turning behavior: Animals with dopaminergic neurons loss below this threshold did not present turning behavior; those that crossed the lower threshold of a partial loss of dopaminergic neurons presented ipsiversive turning behavior; those that crossed the second threshold by losing almost all midbrain dopaminergic neurons presented contraversive turning behavior. Only 6-OHDA rats behaved like that.

The evidences pointed out above describe, but not necessarily explain, why the magnitude of the loss of midbrain dopaminergic neurons determines the direction of turning behavior in rats. Our findings suggest that this explanation comes from the dopaminergic synaptic transmission in the striatum: A partial depletion of dopamine reduced D1 binding in MPTP rats, while severe dopamine depletion increased D2 binding sites in 6-OHDA rats. It makes sense to suppose that it was the modulation of D1 and D2 receptors in the striatum that caused the ipsiversive or contraversive turns in the rats challenged with apomorphine. The dopamine receptors activation in the striatum desinhibit the basothalamic output that stimulates cortical motor areas (Alexander et al. 1986). Turning behavior is caused by a disbalance among ipsilateral and contralateral striatal dopaminergic stimulation (Schwarting and Huston, 1996b).

Dopamine receptor activation in the striatum is schematically presented in Fig. 5. In MPTP rats there is a decrease of dopamine levels and reduce binding to D1 receptors in the lesioned side. Therefore, the non-lesioned side is more stimulated by the endogenous dopamine plus the exogenous dopamine receptor agonist (apomorphine). In 6-OHDA rats there is an increase of D2 receptors in the lesioned side that can compensate the decrease of dopamine – in the presence of apomorphine, the lesioned side is more stimulated than the non-lesioned side. Similar findings have been reported in previous studies of 6-OHDA rats (Thal et al., 1979; Dewar et al., 1997; Newman-Tancredi et al., 2001; Nikolaus et al., 2003; Inaji et al., 2005; Xu et al., 2006) and MPTP monkeys (Alexander et al., 1993). Therefore, the balance of dopamine

neurotransmitter in the lesioned vs. non-lesioned side are the opposite in MPTP and 6-OHDA rats. It would explain the opposite directions of their turning behavior.

Ipsiversive turning behavior of 6-OHDA and MPTP rats challenged with amphetamine (Ungerstedt and Arbuthnott, 1970) can also be explained by this model, as presented in Fig. 5. Since in both MPTP and 6-OHDA rats, amphetamine induces a higher release of dopamine the non-lesioned side, this side is more stimulated in both cases. Therefore, both MPTP and 6-OHDA rats present ipsiversive turning behavior when challenged with amphetamine.

In summary, the present study presents a two-thresholds model to explain the ipsiversive and contraversive turning behavior of unilaterally-lesioned MPTP and 6-OHDA rats. The first threshold is reached by medium-size loss of dopaminergic neurons induced by MPTP. It results in down-regulation of D1 receptors and ipsiversive behavior in apomorphine challenged animals. The second threshold is reached by an almost total loss of dopaminergic lesions induced by 6-OHDA. It causes D2 up-regulation and contraversive turning behavior after a challenge with apomorphine. In the first case the higher stimulation of dopamine receptors in the non-lesioned side resulted in ipsiversive turning behavior. Contraversive turning behavior in the latter case resulted from the higher stimulation of dopamine receptors in the lesioned side.

It is possible that the frontier that separates these two stages is the same that separates the early and the late phases of PD. Nowadays some studies suggest that one of the most marked disability observed in the late phase, dyskinesia, is closed related to contraversive turns in 6-OHDA rats (Lane et al., 2006; Konitsiotis and Tsironis, 2006). Our previous studies presented the MPTP rats as a good model to study cognitive impairments that appear in the early phase of PD (Da Cunha et al., 2001, 2002, 2003; Gevaerd et al., 2001a,b; Miyoshi et al., 2002; Bellissimo *et al*., 2004; Braga et al., 2005; Perry et al., 2005). If this is true, it would be worthy to study MPTP and 6-OHDA rats to better understand the mechanisms that govern the characteristics and limits of the stages of this disease.

REFERENCES

Alexander GE, DeLong MR, Strick, PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Ann Rev Neurosci 9:357–381.

Alexander GM, Schwartzman RJ, Grothusen JR, Brainard I, Gordon SW (1993) Changes in brain dopamine-receptors in mptp parkinsonian monkeys following L-dopa treatment. Brain Res 625:276–282.

Annett LE, Wannerton LA, Watt CA, Thiemann, G, Hasenohrl RU (2006) Behavioral responses directed into contralateral compared with ipsilateral space spare dopamine in the striatum following a unilateral 6-OHDA lesion of the medial forebrain bundle in rats. Cell Transplant 15:549–550.

Bellissimo MI, Kouzmine I, Ferro MM, Oliveira BH, Canteras NS, Da Cunha C (2004) Is the unilateral lesion of the left substantia nigra pars compacta sufficient to induce working memory impairment in rats? Neurobiol Learn Mem 82:150–8.

Blum D, Torch S, Lambeng N, Nissou MF, Benabid AL, Sadoul R, Verna JM (2001) Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson´s disease. Prog Neurobiol 65:135–172.

Braga R, Kouzmine I, Canteras NS, Da Cunha C (2005) Lesion of the substantia nigra, pars compacta impairs delayed alternation in a Y-maze in rats. Exp Neurol 192:134–141.

Costall B, Marsden CD, Naylor RJ, Pycock CJ (1976) Relationship between striatal and mesolimbic dopamine dysfunction and nature of circling responses following 6-hydroxydopamine and electrolytic lesions of ascending dopamine systems of rat-brain. Brain Res 118:87–113.

Da Cunha C, Angellucci MEM, Canteras NS, Wonnacott S, Takahashi RN (2002) The lesion of the rat substantia nigra pars compacta dopaminergic

neurons as a model for Parkinson's disease memory disabilities. Cell Mol Neurobiol 22:227–37.

Da Cunha C, Gevaerd MS, Vital MABF, Miyoshi E, Andreatini R, Silveira R, Takahashi RN, Canteras NS (2001) Memory disruption in rats with nigral lesions induced by MPTP: a model for early Parkinson's Disease amnesia. Behav Brain Res 124:9–18.

Da Cunha C, Wietzikoski S, Wietzikoski EC, Miyoshi E, Ferro MM, Anselmo-Franci JA, Canteras NS (2003) Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system. Neurobiol Learn Mem 79:236–42.

Dewar KM, Paquet M, Reader TA (1997) Alterations in the turnover rate of dopamine D1 but not D2 receptors in the adult rat neostriatum after a neonatal dopamine denervation. Neurochem Int 30:613–621.

Fallon JH, Loughlin SE (1985) Substantia nigra. In: The rat nervous system (Paxinos G, ed), pp353-374. San Diego: Academic Press.

Gardner EL, Mishra RK, Iordanidis P, Katzman R, Makman MH (1976) Catalytic and receptor components of enhanced dopamine-stimulated adenylate-cyclase in 2 behaviorally distinct striatal denervation syndromes. Fed Proc 35:456–456.

Gerlach M, Riederer P (1996) Animal models of Parkinson's disease: An empirical comparison with the phenomenology of the disease in man. J Neural Transm 103:987–1041.

Gevaerd MS, Miyoshi E, Silveira R, Canteras NS, Takahashi RN, Da Cunha C (2001a) L-DOPA restores striatal dopamine level but fails to reverse MPTP-induced memory deficits in rats. Int J Neuropsychoph 4:361–370.

Gevaerd MS, Takahashi RN, Silveira R, Da Cunha C (2001b) Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Res Bull 55:101–106.

Giovanni A, Sieber BA, Heikkila RE, Sonsalla PK (1994) Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine. Part 1.Systemic administration. J Pharmacol Exp Ther 270:1000–1007.

Hirschhorn ID, Hittner D, Gardner EL, Cubells J, Makman MH (1983) Evidence for a role of endogenous opioids in the nigrostriatal system - influence of naloxone and morphine on nigrostriatal dopaminergic super-sensitivity. Brain Res 270:109–117.

Inaji M, Okauchi T, Ando K, Maeda J, Nagai Y, Yoshizaki T, Okano H, Nariai T, Ohno K, Obayashi S, Higuchi M, Suhara T (2005) Correlation between quantitative imaging and behavior in unilaterally 6-OHDA-lesioned rats. Brain Res 1064:136–145.

Jasso-Lopez D, Tapia R (1995) Neurotoxic effect of intranigral injection of 1-methyl-4-phenylpyridinium on gaba-containing neurons and its relation to circling behaviour. J Neurochem 64:794–801.

Kondo J, Kitanura Y, Inden M, Taniguchi T (2004) Hemiparkinsonian rat models: different sensitivity of dopaminergic neurotoxins. Int Congr Ser 1260: 281-285.

Konitsiotis S, Tsironis C (2006) Levodopa-induced dyskinesia and rotational behavior in hemiparkinsonian rats: Independent features or components of the same phenomenon? Behav Brain Res 170:337–341.

Lane EL, Cheetham SC, Jenne P (2006) Does contraversive circling in the 6-OHDA-lesioned rat indicate an ability to induce motor complications as well as therapeutic effects in Parkinson’s disease? Exp Neurol 197:284–290.

Marin C, Rodriguez-Oroz MC, Obeso JA (2006) Motor complications in Parkinson's disease and the clinical significance of rotational behavior in the rat: Have we wasted our time? Exp Neurol 197:269–274.

Miyoshi E, Wietzikoski S, Camplessei M, Silveira R, Takahashi RN, Da Cunha C (2002) Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. Brain Res Bull 58:41–47.

Mori S, Crain BJ, Chacko VP, Van Zijl PCM (1999) Three-Dimensional Tracking of Axonal Projections in the Brain by Magnetic Resonance Imaging. Ann Neurol 45:265–269.

Newman-Tancredi A, Cussac D, Brocco M, Rivet JM, Chaput C, Touzard M, Pasteau V, Millan MJ (2001) Dopamine D receptor-mediated G-protein activation in rat striatum: 2 functional autoradiography and influence of unilateral 6-hydroxydopamine lesions of the substantia nigra. Brain Res 920:41–54.

Nicola SM, Surmeier DT, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Ann Rev Neurosci 23:185–215.

Nikolaus S, Larisch R, Beu M, Vosberg H, Muller-Gartner HW (2003) Bilateral increase in striatal dopamine D-2 receptor density in the 6-hydroxydopamine-lesioned rat: a serial in vivo investigation with small animal pet. Eur J Nucl Med Mol Imaging 30:390–395.

Nobrega JN, Richter A, Tozman N, Jiwa D, Loscher W (1996) Quantitative autoradiography reveals regionally selective changes in dopamine D1 and D2 receptor binding in the genetically dystonic hamster. Neuroscience 71:927–936.

Olds ME, Jacques DB, Kopyov O (2006) Relation between rotation in the 6-OHDA, lesioned rat and dopamine loss in striatal and substantia nigra subregions. Synapse 59:532–544.

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates, Ed 5. San Diego: Academic Press.

Perry JC, Hipólide DC, Tufik S, Martins RD, Da Cunha C, Andreatini R, Vital MABF (2005) Intra-nigral MPTP lesion in rats: behavioral and autoradiography studies. Exp Neurol 195:322–329.

Redgrave P, Mitchell I (1982) Functional validation of projection topography in the nigrostriatal dopamine system. Neuroscience 7:885–894.

Sasaki T, Kennedy JL, Nobrega JN (1997) Localized changes in the GABA-gated chloride channel in rat brain after long-term haloperidol: relation to vacuous chewing movements. Synapse 25:73–79.

Schwarting RK, Huston JP (1996a) Unilateral 6-hydroxydopamine lesions of the meso-striatal dopaminergic neurons and their physiological sequelae. Prog Neurobiol 49:215–266.

Schwarting RK, Huston JP (1996b) The unilateral 6-hydroxydopamine lesion model in behavioral brain research: analysis of functional deficits, recovery and treatments. Prog Neurobiol 50:275–331.

Sindhu KM, Banerjee R, Senthilkumar KS, Saravanan KS, Raju BC, Rao JM, Mohanakumar KP (2006) Rats with unilateral median forebrain bundle, but not striatal or nigral, lesions by the neurotoxins MPP+ or rotenone display differential sensitivity to amphetamine and apomorphine. Pharmacol Biochem Behav 84:321–329.

Takeda R, Ikeda T, Tsuda F, Abe H, Hashiguchi H, Ishida Y, Nishimori Y (2005) Unilateral lesions of mesostriatal dopaminergic pathway alters the withdrawal response of the rat hindpaw to mechanical stimulation. Neurosci Res 52:31–36.

Thal L, Mishra RK, Gardner EL, Horowitz SG, Varmuza S, Makman MH (1979) Dopamine antagonist binding increases in 2 behaviorally distinct striatal denervation syndromes. Brain Res 170:381–386.

Truong L, Allbutt H, Kassiou M, Henderson JM (2006) Developing a preclinical model of Parkinson's disease: A study of behaviour in rats with graded 6-OHDA lesions. Behav Brain Res 169:1–9.

Ungerstedt U (1968). 6-hydroxydopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5:107–110.

Ungerstedt U (1971) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of nigro-striatal dopamine system. Acta Physiol Scand 367:69–93.

Ungerstedt U, Arbuthnott GW (1970). Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. Brain Res 24:485–493.

Xu ZC, Ling G, Sahr RN, Neal-Beliveau BS (2005) Asymmetrical changes of dopamine receptors in the striatum after unilateral dopamine depletion. Brain Res 1038:163–170.

Yuan H, Sarre S, Ebinger G, Michotte Y (2005) Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. J Neurosci Meth 144:35–45.

FIGURE LEGENDS

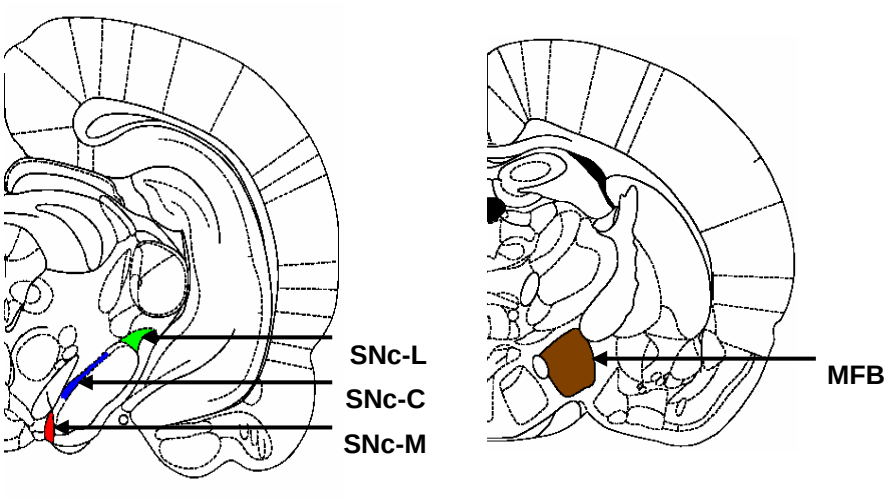

Figure 1: Schematic drawing of coronal slices of the rat brain showing the sites of injection of MPTP or 6-OHDA. Adapted from Paxinos and Watson (2005).

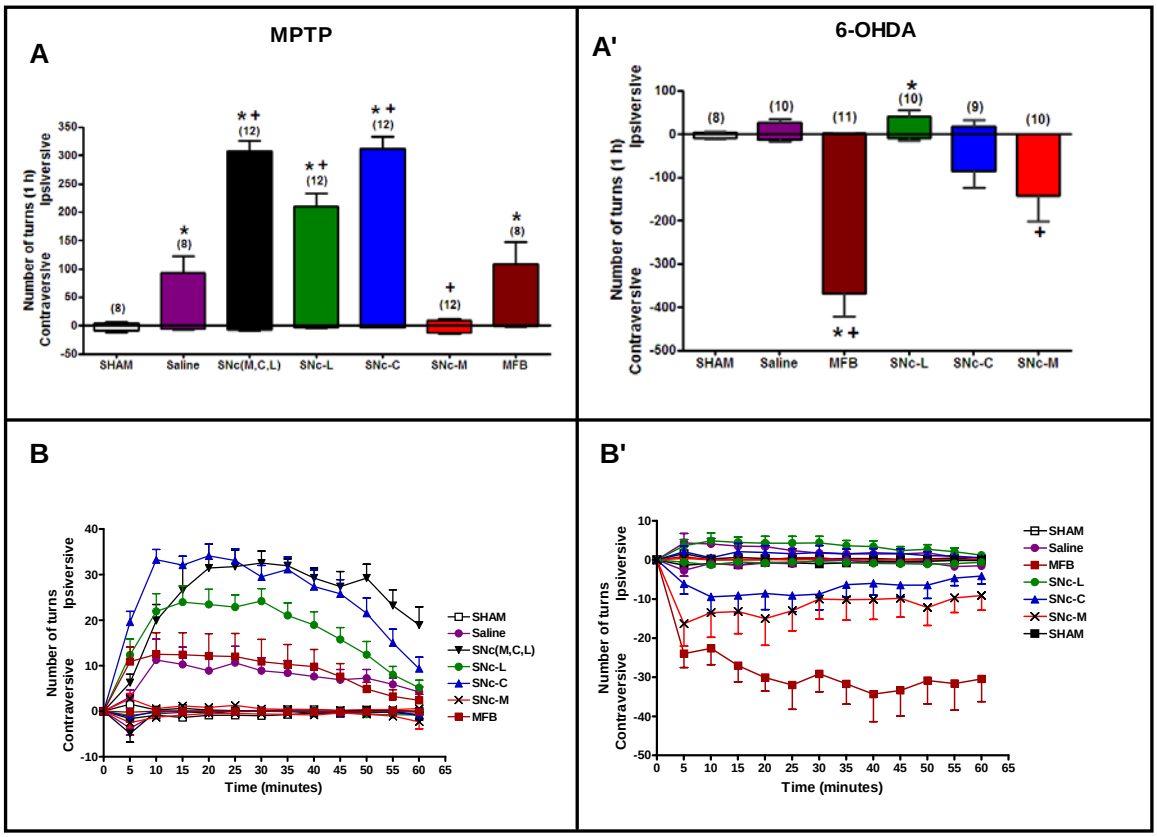

Figure 2: Turning behavior of MPTP and 6-OHDA unilaterally lesioned rats challenged with apomorphine. Data expresses the number of ipsiversive (positive scale) and contraversive turns (negative scale) counted during the 1 hour after the drug challenge (up) or counted in intervals of 5 min (lower). The number animals per group are printed above the bars. * P < 0.05 compared to sham group; + P < 0.05 compared to saline group. Newman Keuls after one-way ANOVA.

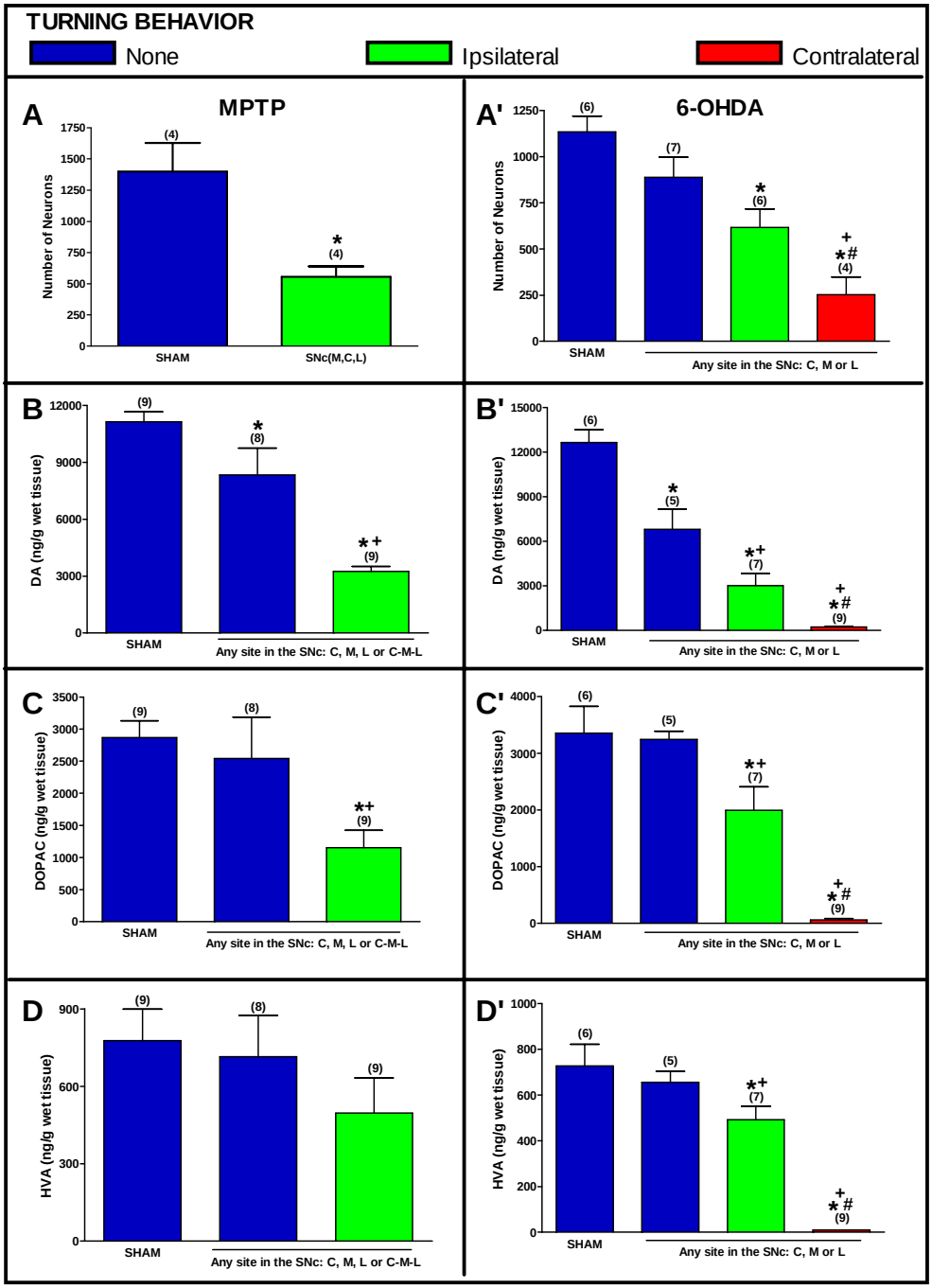

Figure 3: Data expresses the number of TH-immunostained neurons, dopamine, DOPAC, and HVA in the ipsilateral mesecephalon of MPTP and 6-OHDA unilaterally lesioned or sham rats. The animals were grouped by their turning behavior as indicated by the color of the bars. The number animals per group are printed above the bars. * P < 0.05 compared to sham group, Newman

Keuls after one-way ANOVA. DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovallinic acid.

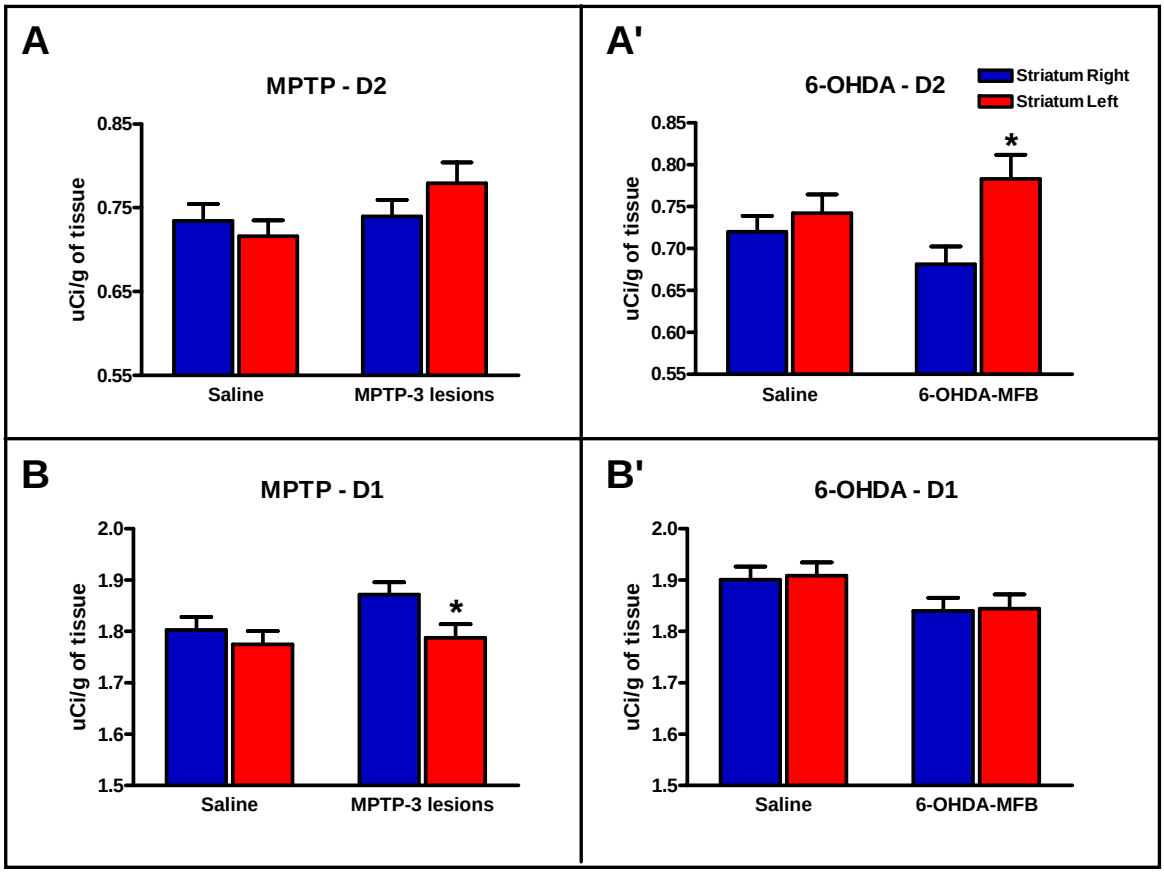

Figure 4: Binding of [3H]raclopride [3H]SCH-23390 to striatal slices of rats one week after MPTP or 6-OHDA administration. The values are expressed as means ± SEM of uCi/g tissue. * $P < 0.05$, compared to the non-lesioned side.

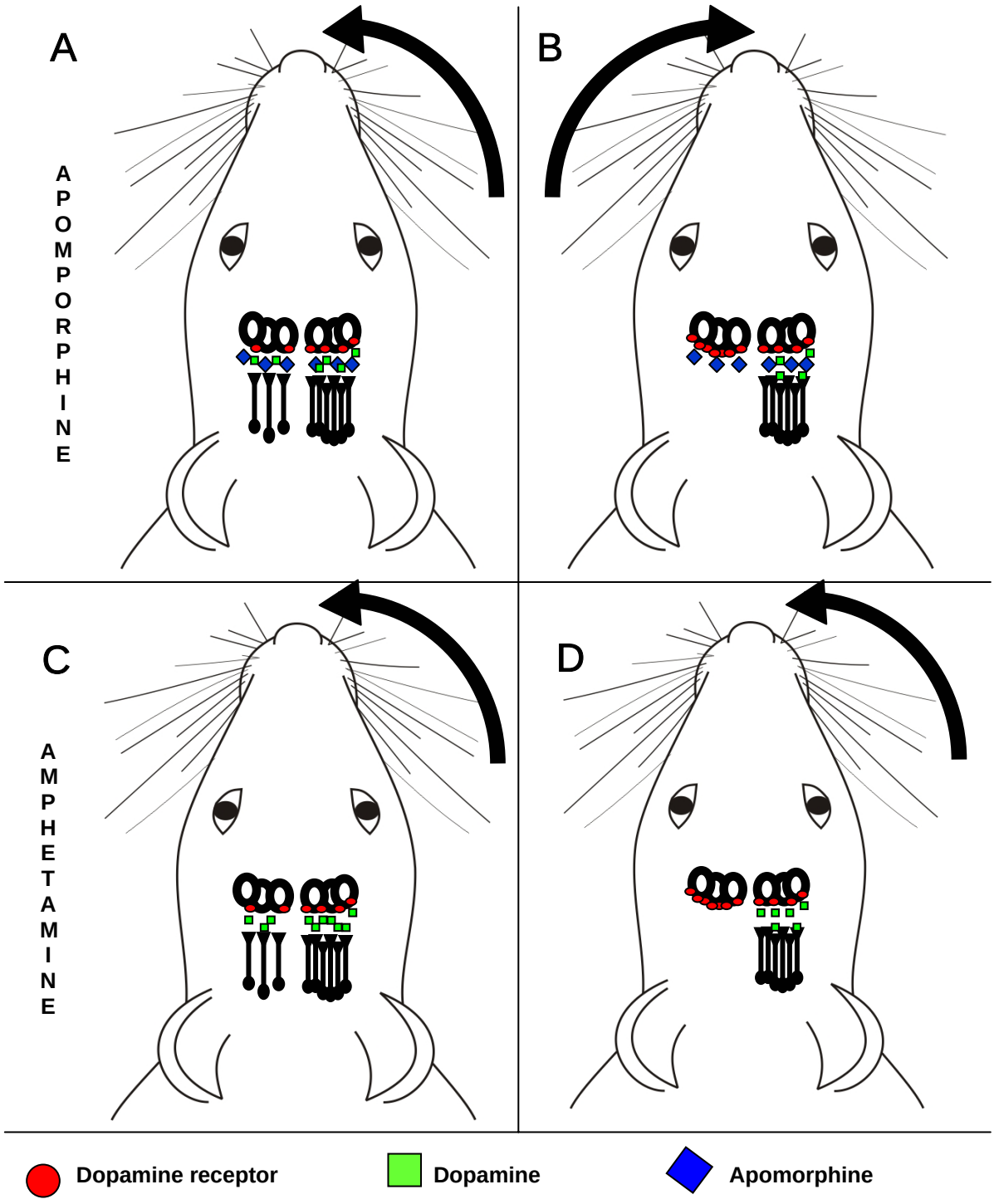

Figure 5: The figure represent rats with partial (A, C) or total (B, D) loss of nigrostriatal dopamine receptor neurons, challenged with apomorphine (A, B) or amphetamine (C,D). The animal rotates towards the site that present the weaker synaptic signal. The synaptic efficacy depends of the amount of dopamine receptor agonists (dopamine + apomorphine) and of the density of dopamine receptorsin the striatal neurons. See discussion for details.

4 DISCUSSÃO

O screening de drogas com ação sobre o sistema dopaminérgico tem evoluído consideravelmente após a introdução do teste do comportamento rotatório em animais com lesão unilateral por 6-OHDA (UNGESTEDT, 1968), o qual reproduz o estágio final da DP. Os animais lesados com 6-OHDA no FPM apresentam uma perda de todos os neurônios dopaminérgicos mesencefálicos com uma depleção total nos níveis de DA neoestriatal (KONDOH *et al*, 2005; SCHWARTING e HUSTON, 1996), semelhante ao que ocorre em pacientes com DP em uma fase bastante adiantada (BRAAK *et al.*, 2003). Entretanto, um modelo de desnervação parcial da via nigroestriatal poderia mimetizar a fase inicial da doença, sendo satisfatória para testar novas drogas. A lesão por MPTP causa uma redução parcial de neurônios dopaminégicos e da DA estriatal (FERRO *et al.*, 2005; GEVAERD *et al.*, 2001a; Da CUNHA *et al.*, 2001; HARIK *et al.*, 1987), podendo ser utilizado como um modelo que reproduz a fase inicial da DP.

Neste trabalho foi comparado o comportamento rotatório de dois modelos animais da DP: o modelo de lesão da via nigroestriatal com 6-OHDA, utilizado para o estudo das alterações motoras da DP e ação de drogas antiparkinsonianas (LANE *et al*., 2006; UNGESTEDT,1968, 1970), e o modelo de lesão da via nigroestriatal com MPTP, utilizado para o estudo dos déficits cognitivos (Da CUNHA *et al.*, 2006, 2003; MIYOSHI *et al.*, 2002). Estudo realizado por Ferro *et al.*, 2005 demonstrou diferenças entre os dois modelos, comparações histológicas e neuroquímicas sugeriram que a lesão por 6-OHDA é muito mais efetiva comparada com a produzida pelo MPTP.

No experimento 1, a proposta do estudo foi validar um modelo animal que pudesse reproduzir os estágios iniciais da DP, caracterizado pela perda parcial de neurônios dopaminérgicos, o qual pudesse ser utilizado para testar novas abordagens terapêuticas para este estágio da doença.

O modelo de lesão unilateral produzido por 6-OHDA já está bem caracterizado para o estudo do comportamento rotatório induzido pelo desafio de agonista direto, a apomorfina, e indireto, a anfetamina (MARIN *et al.*, 2006;

UNGESTEDT,1968, 1970). Entretanto, outro modelo também poderia ser utilizado, o do MPTP. Neste trabalho, os ratos com lesão unilateral da SNc produzida pelo MPTP e desafiados com apomorfina ou com anfetamina apresentaram comportamento rotatório dose-dependente ipsilateral a lesão. A explicação clássica para este comportamento é de que ocorre um desequilíbrio dopaminérgico entre o lado lesado e não-lesado mediado através da ativação do terminal nigroestriatal não-lesado, ocasionando a rotação ipsilateral. Na literatura é reportado que rotações ipsilaterais podem ser detectadas em animais que tiveram até 50% de lesão, e ainda, tem sido aplicada para avaliar a viabilidade de enxertos neurais e tratamentos neuroprotetores (CASTAÑEDA *et al.*, 2005; WINKLER *et al.*, 1999; SCHWARTING e HUSTON, 1996; CARMAN *et al.*, 1991). Por outro lado, rotações contralaterais induzidos pela levodopa ou apormorfina, são devido à estimulação de receptores dopaminérgicos supersensibilizados no estriado desnervado, o qual ocorre somente quando aproximadamente >90% dos neurônios dopaminérgicos da SNc foram eliminados (DEUMENS *et al.*, 2002; HEFTI *et al.*, 1980). Alguns autores sugerem que as rotações contralaterias podem não representar somente uma atividade antiparkinsoniana, mas também estar correlacionada com discinesia (KONITSIOTIS e TSIRONIS, 2006; LANE *et al.*, 2006).

Os resultados apresentados neste estudo validam o comportamento rotatório ipsilateral de animais com lesão unilateral por MPTP como um modelo simples e quantitativo, com valor preditivo, para screening de drogas efetivas nos prejuízos motores observados na fase inicial da DP.

No experimento 2, a proposta foi tentar explicar qual é o fator crítico para o sentido das rotações. Na literatura, para trabalhar com amostras mais homogêneas, muitos autores excluem de seus estudos, animais que apresentam comportamento rotatório ipsilateral a lesão (MARIN *et al.,* 2006 LANE *et al.,* 2006; TAKEDA *et al.*, 2005; KONDOH *et al.*, 2005). No nosso trabalho, não descartamos nenhum animal devido ao seu comportamento, ao contrário, tentamos entender qual o fator crítico que determina o sentido das rotações de animais com lesão unilateral por 6-OHDA ou MPTP. Os resultados deste estudo sugerem que existem diferenças quanto ao mecanismo das neurotoxinas MPTP e 6-OHDA sobre o comportamento rotatório dos animais.

No presente trabalho testamos a hipótese de que a região de infusão da neurotoxina influência o sentido da rotação. Para tanto, foram administrados MPTP e 6-OHDA nas regiões lateral, central e medial da SNc e no FPM. Os animais lesados unilateralmente com MPTP, com exceção do grupo lesado na porção medial da SNc, que se comportou semelhante ao grupo SHAM, apresentaram predominantemente um comportamento rotatório ipsilateral a lesão, sendo que o sentido rotatório dos animais se mantém independentemente da região de infusão da neurotoxina. A porção medial da SNc projeta fibras para as regiões mediais do estriado (GERFEN, 2004), provavelmente a lesão feita nesta região provoca uma lesão mais branda nos neurônios dopaminérgicos, o que pode estar influenciando na intensidade com que os animais rodam o que justificaria o seu comportamento semelhante aos animais do grupo SHAM.

Os animais neste trabalho infundidos unilateralmente com 6-OHDA na SNc se comportaram rodando contralateral ou ipsilateral a lesão. É citado na literatura que diferentes tamanhos (SCHWARTING e HUSTON, 1996) e locais de lesão (HIRSCHHORN *et al.*, 1983) podem influenciar a intensidade e o sentindo da rotação. Quando os animais lesados na SNc com 6-OHDA foram agrupados de acordo com o comportamento rotatório, foi observado que os animais com rotações contralaterais à lesão perderam uma maior quantidade de neurônios dopaminérgicos do que os animais que se comportaram com rotações ipsilaterais; estes últimos perderam mais neurônios dopaminégicos que os animais assintomáticos.

O comportamento rotatório nos dois casos parece guardar uma relação com o número total de neurônios dopaminérgicos que foram perdidos, ou seja, o tamanho/magnitude da lesão na SNc tem um efeito maior sobre o sentido das rotações induzidas por apomorfina do que a região de administração das neurotoxinas na SNc. O número de rotações observadas após a administração de um agonista ou antagonista dopaminérgico pode distinguir entre uma lesão da SNc parcial e uma lesão próxima da desnervação completa (> 90%) (SINDHU *et al.*, 2006). Porém, lesões menores de 50 – 80% resultam em comportamento rotatório menos expressivo. Esta hipótese pôde também ser confirmada pelos resultados neuroquímicos (depleção de dopamina e

metabólitos) neste trabalho, em consonância com outros trabalhos da literatura (YUAN *et al.*, 2005).

O fator crítico que determina um comportamento rotatório contralateral parece estar mais na magnitude da perda de neurônios dopaminégicos e dopamina que na área onde houve perda destes neurônios (mesmo que parcial). Os animais lesados com 6-OHDA no FPM apresentam uma perda de todos os neurônios dopaminérgicos mesencefálicos com uma depleção total nos níveis de DA neoestriatal (KONDOH *et al.*, 2005; SCHWARTING e HUSTON, 1996), quando desafiados com apomorfina apresentam um comportamento rotatório contralateral a lesão como foi observado em nossos resultados. Entretanto, os animais lesados com MPTP simultaneamente nos três sítios da SNc que também representa uma lesão extensa, se comportaram de forma diferente, rodando ipsilateral a lesão. A análise histológica demonstrou que a lesão provocada pela 6-OHDA injetada no FPM ocasiona morte de todos os neurônios dopaminérgicos da SNc, inclusive a ATV, ao contrário nos animais com três lesões simultâneas na SNc por MPTP houve perda de neurônios nesta área, sem no entanto atingir a ATV. Os resultados da neuroquímica também demonstram que estes animais apresentam uma depleção de aproximadamente 50% da DA estriatal. Já os animais lesados com 6-OHDA no FPM apresentam uma grande depleção nos níveis de DA e seus metabólitos maior que 90%.

Estes resultados sugerem que o tamanho da lesão unilateral da SNc parece ser o principal fator para definir o sentido da rotação dos ratos desafiados com apomorfina. Provavelmente uma lesão muito extensa e total causa uma depleção crônica de dopamina estriatal que induz uma supersensibilidade dos receptores dopaminérgicos a apomorfina, causando rotações contralaterais (SCHWARTING e HUSTON, 1996). Esta lesão quase total em ratos só pode ser produzida com 6-OHDA, mas não com MPTP. No caso da lesão total com supersensibilidade dos receptores pós-sinápticos, a apomorfina estimula mais os receptores supersensibilizados do lado lesado, resultando em rotações contralaterais. No caso da lesão parcial sem supersensibilidade, o lado lesado fica com menos dopamina e com os receptores normais, então o estímulo apoformina + dopamina é maior no lado

não lesado que no lado lesado, o que explica as rotações no sentido ipsilateral à lesão.

Os dados da autoradiografia demonstraram que nos animais lesados no FPM com 6-OHDA houve superexpressão de receptores D2 no lado lesado comparado ao lado não-lesado, diferente dos animais com três lesões na SNc por MPTP. Não houve alteração na expressão de receptores D1. Já os animais lesados com MPTP, mesmo aqueles que receberam a infusão da neurotoxina em 3 sítios na SNc, houve uma redução na expressão de receptores D1 e não houve alteração em D2. Isto explica porque os primeiro apresentaram rotações contralaterais e os sengundos, ipsilaterais.

Portanto, a lesão por MPTP em ratos pode estar reproduzindo o fenômeno que ocorre quando pacientes com DP em estágio inicial são tratados com drogas dopaminérgicas. O comportamento rotatório ipsilateral de animais com lesão por MPTP, oposto ao que ocorre em animais com lesão por 6-OHDA pode ser um método simples e quantitativo para obter resultados que podem auxiliar na seleção de drogas úteis para o tratamento da fase inicial da DP.

5 CONCLUSÔES

- No modelo de lesão unilateral por MPTP, os agonistas dopaminérgicos direto e indireto induzem comportamento rotatório dose-dependente ipsilateral à lesão. Este resultado sugere que este modelo pode ser utilizado para testar novas abordagens terapêuticas para a fase inicial da DP.
- O sentido das rotações após um desafio com apomorfina não depende da região onde foi infundida a neurotoxina na SNc, mas sim do tamanho/magnitude da lesão na SNc avaliada pela contagem de neurônios dopaminérgicos mesencefálicos ou pela dosagem de dopamina e metabólitos estriatais. Uma lesão parcial produz rotações ipsilaterais e uma lesão quase total produz rotações contralaterais.
- A diferença no comportamento rotatório que ocorre em animais com lesão por MPTP ou por 6-OHDA parece estar relacionada à supersensibilização de receptores dopaminérgicos no estriado, que ocorre somente em animais com uma lesão quase total por 6-OHDA. O MPTP em ratos não produziu lesão total, supersensibilização de receptores dopaminérgicos, nem comportamento rotatório contralateral.

6 REFERÊNCIAS BIBLIOGRÁFICAS

AGID, Y.; RUBERG, M.; RAISMAN-VOZARI, R.; HIRSCH, E.C.; JAVOY-AGID, F. The biochemistry of Parkinso´s disease. In: Stern, GM (Ed.),Parkinson´s disease. Chapman & Hall, London, pp. 99-125, 1990.

ALEXANDER, G.E.; CRUTCHER, M.D. Functional architecture of basal ganglia circuits - neural substrates of parallel processing. Trends Neurosciences. v.13, p. 266-271, 1990.

ALEXANDER, G.E.; DeLONG, M.R.; STRICK, P.L. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annual Review of Neuroscience. v.9, p. 357-381, 1986.

BEAL, M.F. Experimental models of Parkinson´s disease. Nature Reviews. v.2, p. 325-332, 2001.

BELLISSIMO, M. I.; KOUZMINE, I.; FERRO, M..; OLIVEIRA, B.H.; CANTERAS, N. S.; Da CUNHA, C. Is the unilateral lesion of the substantia nigra pars compacta sufficient to induce working memory impairment in rats? Neurobiology of Learning and Memory. v. 82, p.150-158, 2004.

BETARBET, R.; SHERER, T.B.; GREENAMYRE, J.T. Animal models of Parkinson´s disease. Bioessays. v. 24, p. 308-318, 2002.

BEZARD, E.; GROSS, C. E.; FOURNIER, M. C.; DOVERO, S.; BLOCH, B.; JABER, M. Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter. Experimental Neurology. v.155, p.268-273, 1999.

BLUM, D.; TORCH, S.; LAMBENG, N.; NISSOU, M. F.; BENABID, A. L.; SADOUL, R.; VERNA, J. M. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and

MPTP: contribution to the apoptotic theory in Parkinson´s disease. Progress in Neurobiology, v.65, p. 135-172, 2001.

BOGERTS, B. A brainsteim atlas of catecolaminergic neurons in man, using melanin as a natural marker. Journal Comparative Neurology. v. 197, p. 63-80, 1981.

BRAAK, H.; DEL TREDICI, K.; RUB, U.; DE VOS, R.A.I.; STEUR, E.N.H.J.; BRAAK, E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiology of Aging, v. 24, p.197-211, 2003.

BRAGA, R.; KOUZMINE, I.; CANTERAS, N.; DA CUNHA, C. Lesion of the substantia nigra, pars compacta impairs delayed alternation in a Y-maze in rats. Experimental Neurology. v.192, p. 134-141, 2005.

BRENNER, H.; ARNDT, V. Epidemiology in aging research. Experimental Gerontology, v.39, p. 679–686, 2004.

CANDY, J.M.; PERRY, R. H.; PERRY, E.K. IRVING, D.; BLESSED, G.; FAIRBAIRN, A. F.; TOMLINSON, B. E. Pathological changes in the nucleus of Meynert in Alzheimer´s and Parkinson´s diseases. Journal Neurology Science. v. 59, p. 277-289, 1983.

CARMAN, L. S.; GAGE, F. H.; SHULTS, C. W. Partial lesion of the substantia nigra: relation between extent of lesion and rotational behavior. Brain Research, v.553, p.275–283, 1991.

CASTAÑEDA, E.; FLEMING, S.; PAQUETTE, M. A.; BOAT, K.; MOFFETT, J.; STACHOWIAK, E. K.; BLOOM, D. C.; STACHOWIAK, M. K. Assessment of recovery in the hemiparkinson rat: Drug-induced rotation is inadequate. Physiology & Behavior, v.84, p. 525–535, 2005.

CENCI, M.A.; WHISHAW, I.Q.; SCHALLERT, T. Animal models of neurological deficits: how relevant is the rat? Nature Reviews. v.3, p. 574-579, 2002.

Da CUNHA, C.; ANGELUCCI, M.E.M.; CANTERAS, N.S.; WONNACOTT, S.; TAKAHASHI, R.N. The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson's disease memory disabilities. Cellular and Molecular Neurobiology, v. 22, p. 227-237, 2002.

Da CUNHA, C.; GEVAERD, M. S.; VITAL, M.A.B.F.; MIYOSHI, E.; ANDREATINI, R.; SILVEIRA, R.; TAKAHASHI, R. N.; CANTERAS, N.S. Memory disruption in rats with nigral lesions induced by MPTP: a model for early Parkinson's disease amnesia. Behavioural Brain Research. v.124; p. 9-18, 2001.

Da CUNHA, C.; SILVA, M.H.C.; WIETZIKOSKI, S.; WIETZIKOSKI, E.; FERRO, M.S.; KOUZMINE, I.; CANTERAS, N.S. Place learning strategy of substantia nigra pars compacta-lesioned rats. Behavioral Neuroscience, 2006, in press.

Da CUNHA, C.; WIETZIKOSKI, S.; WIETZIKOSKI, E.; FERRO, M.; MIYOSHI, E.; ANSELMO-FRANCI, J.A.; CANTERAS, N.S. Evidence for the substantia nigra pars compacta as an essential component of a memory system independent of the hippocampal memory system. Neurobioloby of Learning and Memory. v. 79, p. 236-242, 2003.

DAUER, W.; PRZEDBORSKI, S. Parkinson´s disease: mechanisms and models. Neuron. v.39, p.889-909. 2003.

DEL TREDICI, K.; RÜB, U.; VOS, R. A. I.; BOHL, J. R. E.; BRAAK, H. Where Does Parkinson Disease Pathology Begin in the Brain? Journal of Neuropathology and Experimental Neurology, v.61, p. 413- 426, 2002.

DELEU, D.; NORTHWAY, M.G.; HANSSENS, Y. Clinical pharmacokinetic and pharmacodynamic properties of drugs used in the treatment of Parkinson´s disease. Clinical Pharmakocinetic. v.41, p. 261-309, 2002.

DEUMENS, R.; BLOKLAND, A.; PRICKAERTS, J. Modeling Parkinson's disease in rats: An evaluation of 6-OHDA lesions of the nigrostriatal pathway. Experimental Neurology. v. 175, p. 303-317, 2002.

DUNNET, S. B.; BJÖRKLUND, A. Functional neural transplantation. Raven, New York, 1994.

EMBORG, M. E. Evaluation of animal models of Parkinson´s disease for neuroprotective strategies. Journal of Neuroscience Methods. v.139, p.121-143, 2004.

FALLON, J.H.; LOUGHLIN, S.E. The rat nervous system. Academic Press Australia, Australia. Cap.9, 353-374, 1985.

FEARNLEY, J.; LESS, A.J. Parkinson´s disease: Neuropatology. Brain. v.114, p. 2283-2301, 1991.

FERRO, M.; BELLISSIMO, M.I.; ANSELMO-FRANCI, J.A.; ANGELUCCI, M.E.M.; CANTERAS, N.S.; DA CUNHA, C. Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: Histological, neurochemical, motor and memory alterations, Journal of Neuroscience Methods, v. 148, p. 78-87, 2005.

FLINT, M. B. Experimental models of Parkinson´s disease. Nature rewiews. v.2, p.325-332, 2001.

FORNO, L.S. Neuropathology of Parkinson´s disease. Journal of Neuropathology Experimental Neurology. v. 55, p. 259-272, 1996.

GANONG, W.F. Fisiologia Médica. Rio de Janeiro: 17º ed. Prentice-Hall do Brasil, 578pp, 1995.

GERFEN, C.R. Basal ganglia. In: PAXINOS, G. The rat nervous system. 3th edition, Academic press, Australia. 2004.

GERLACH, M.; RIEDERER, P. Animal models of Parkinson´s disease: an empirical comparison with the phenomenology of the disease in man. Journal of Neural Transmission. v.103, p.987-1041. 1996.

GEVAERD, M.S.; MIYOSHI, E.; SILVEIRA, R.; CANTERAS, N.S.; TAKAHASHI, R.N.; Da CUNHA, C. Levodopa treatment restores the striatal level of dopamine but fails to reverse memory deficits in rats treated with MPTP, an animal model of Parkinson's disease. International Journal of Neuropsychopharmacology. v. 4, p. 361-370, 2001a.

GEVAERD, M.S.; TAKAHASHI, R.N.; SILVEIRA, R.; Da CUNHA, C. Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Research Bulletin. v. 55, p. 101-106, 2001b.

GIOVANNI, A.; SIEBER, B.A.; HEIKKILA, R.E.; SONSALLA, P.K. Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine. Part 1.Systemic administration. Journal of Pharmacology Experimental Therapy. v.270, p.1000-1007, 1994a.

GIOVANNI, A.; SONSALLA, P.K.; HEIKKILA, R.E. Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine. Part 2. Central administration of 1-methyl-4phenylpyridinium. Journal of Pharmacology Experimental Therapy. v.270, p.1008-1014, 1994b.

GLINKA, Y.; GASSEN, M.;YOUDIM, M.B. Mechanism of 6-hidroxydopamine neurotoxicity. Journal of Neural Trasmission Supplementar. v.50, p.55-66, 1997.

GOTO, S.; HIRANO, A.; MATSUMOTO, S. Subdivisional involvement of nigrostriatal loop in idiopathic Parkinson´s disease and striatonigral degeneration. Annalogy Neurology. v.26, p. 766-770, 1989.

GRAHAM, D. G. Oxidative pathways for catecolamines in the gênesis of neuromelanin and cytotoxic quinones. Molecular Pharmacology. v.14, p. 933-643, 1978.

HALBACH, O.B.; SCHOBER, A.; KRIEGLSTEIN, K. Genes, proteins, and neurotoxins involved in Parkinson´s disease. Progress in Neurobiology. v.73, p. 151-177, 2004.

HARIK, S.I., SCHMIDLEY, J.W., IACOFANO, L.A., BLUE, P., ARORA, P.K.; SAYRE, L.M. On the mechanisms underlying 1-methyl-4phenyl-1,2,3,6-tetrahydropiridine neurotoxicity: the effect of perinigral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine, its metabolite and their analogs in rat. Journal of Pharmacology Experimental and Therapeutic, vol. 241, p.669–676, 1987.

HEFTI, F.; MELAMED, E.; SAHAKIAN, B.J.; WURTMAN, R.J. Circling behavior in rats with partial, unilateral nigrostriatal lesions: effect of amphetamine, apomorphine, and L-Dopa. Pharmacology and Biochemistry Behavioral, v.12, p.185–188, 1980.

HEIKKILA, R. E.; SONSALLA, P. K.; DUVOISIN, R.C. Biochemical models of Parkinson's disease. In Neuromethods, Drugs as Tools in Neuroscience Research. v. 12, p. 351-384, 1989.

HIRSCH, E.; GRAYBIEL, A.M.; AGID, Y.A. Melanized dopaminérgico neurons are differentially susceptible to degeneration in Parkinson´s disease. Nature. v. 334, p. 345-348, 1988.

HIRSCHHORN, I.D.; HITTNER, D.; GARDNER, E.L.; CUBELLS, J.; MAKMAN, M.H. Evidence for a role of endogenous opioids in the nigrostriatal system: influence of naloxone and morphine on nigrostriatal dopaminergic supersensitivity. Brain Research. v.270, p.109-117, 1983.

JELLINGER, K. The pathology of parkinsonism. Moviment disorders. v. 2, p. 124-165, 1987.

JONSSON, G.; SACHS, C. Actions of 6-hidroxydopamine quinines on cathecolamine neurons. Journal of Neurochemistry. v. 25, p. 509-516, 1975.

KAHLE, P.J.; HAASS, C.; KRETZSCHMAR, H.A.; NEUMANN, M. Structure/function of alpha-synuclein in health and disease: rational development of animal models for Parkinson´s and related disease. Journal of Neurochemistry. v. 82, p. 449-457, 2002.

KONDO, J., KITAMURA, Y., INDEN, M., TANIGUCHI, T. Hemiparkinsonian rat models: different sensitivity of dopaminergic neurotoxins. International Congress Series. v.1260, p. 281-285, 2004.

KONDOH, T.; BANNAI, M.; NISHINO, H.; TORII, K. 6-Hydroxydopamine-induced lesions in a rat model of hemi-Parkinson's disease monitored by magnetic resonance imaging. Experimental Neurology. v.192, p.194-202, 2005.

KONITSIOTIS, S.; TSIRONIS, C. Levodopa-induced dyskinesia and rotational behavior in hemiparkinsonian rats: Independent features or components of the same phenomenon? Behavioural Brain Research, v. 170, p. 337-341, 2006.

LANE, E. L.; CHEETHAM, S .C.; JENNE, P. Does contraversive circling in the 6-OHDA-lesioned rat indicate an ability to induce motor complications as well as therapeutic effects in Parkinson's disease? Experimental Neurology. v. 197, p. 284-290, 2006.

LANGSTON, J.W. The etiology of Parkinson´s disease with emphasis on the MPTP story. Neurology. v. 47, p. 153-160, 1996.

LANGSTON, J.W.; BALLARD, P.; TETRUD, J. W.; IRWIN, I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science. v.219, p.979-980, 1983.

LENT, R. Cem bilhões de neurônios – Conceitos fundamentais de neurosciência. São Paulo. Editora Atheneu, 2001. 698p.

MARIN, C.; RODRIGUEZ-OROZ, M.C.; OBESO, J.A. Motor complications in Parkinson's disease and the clinical significance of rotational behavior in the rat: Have we wasted our time? Experimental Neurology, v. 197, p. 269–274, 2006.

McGEER, P.L.; ITAGAKI, S.; AKIYAMA, H.; McGEER, E. G. Rate of cell death in parkinsonism indicates active neuropathological process. Annals of Neurology. v. 24, p. 574-576, 1989.

MENESES, M.S.; TEIVE, H. A. G. Doença de Parkinson. Rio de Janeiro: 1º ed. Guanabara Koogan, 348pp, 2003.

METZ, G.A., WHISHAW, I. Q. Drug-induced rotation intensity in unilateral dopamine-depleted rats is not correlated whit end point or qualitative measures of forelimb or hindlimb motor performance. Neuroscience. v. 3, p. 325-336, 2002.

MIYOSHI, E.; WIETZIKOSKI, S.; CAMPLESSEI, M.; SILVEIRA, R.; TAKAHASHI, R.N.; Da CUNHA, C. Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. Brain Research Bulletin. v. 58, p. 41-47, 2002.

NICKLAS, W. J.; VYAS, I.; HEIKKILA, R. E. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4phenylpyridine, a metabolite of the neurotoxin, 1-methyl-4phenyl-1,2,5,6-tetrahydropyridine. Life science. v. 36, p.2503-2508, 1985.

SCHWARTING, R. K. W.; HUSTON, J. P. Unilateral 6-hidroxydopamine lesions of meso-estriatal dopamine neurons and their physiological sequelae. Progress in Neurobiology. vol.49, p. 215-266, 1996.

SCHWARTING, R.K., BONATZ, A.E., CAREY, R.J., HUSTON, J.P., Relationships between indices of behavioral asymmetries and neurochemical changes following mesencephalic 6-hydroxydopamine injections. Brain Research. V. 554, p. 46– 55, 1991.

SINDHU, K. M.; BANERJEE, R.; SENTHILKUMAR, K. S.; SARAVANAN, K. S.; RAJU, B. C.; RAO, J. M.; MOHANAKUMAR, K P. Rats with unilateral median forebrain bundle, but not

striatal or nigral, lesions by the neurotoxins MPP+ or rotenone display differential sensitivity to amphetamine and apomorphine. Pharmacology, Biochemistry and Behavior, v. 84, p. 321–329, 2006.

SINDHU, K.M.; SARAVANAN, K.S.; MOHANAKUMAR, K.P. Behavioral differences in a rotenone-induced hemiparkinsonian rat model developed following intranigral or median forebrain bundle infusion. Brain Research. V.1051, p.25-34, 2005.

SMEYNE, R. J.; JACKSON-LEWIS, V. The MPTP model of Parkinson's disease. Molecular Brain Research. Vol.134, p.57-66, 2005.

SONSALLA, P.K.; ZEEVALK, G. D.; MANZINO,L.; GIOVANNI, A.;NICKLAS, W.J. MK-801 fails to protect against the dopaminérgico neurophatology produced by systemic 1-methyl-4phenyl-1,2,3,6-tetrahidropyridine in mice or intranigral 1-methyl-4-phenylpyridinium in rats. Journal of Neurochemistry, vol. 58, p. 1979-1982, 1992.

STAAL, R.G.; SONSALLA, P. K. Inhibition of brain vesicular monoamine transporter (VMAT2) enhances 1-methyl-4phenylpyridinium neurotoxicity in vivo in rat striata. Journal of Pharmacology Experimental Therapy, vol.293, p.336-342, 2000.

STOREY, E.; HYMAN, B.T.; JENKINS, B.; BROUILLET, E.; MILLER, J.M.; ROSEN, B.R.; BEAL, M.F. 1-methyl-4phenypyridinium produces excitotoxic lesions in rat striatum as a result of impairment of oxidative metabolism. Journal of Neurochemistry, vol.58, p.1975-1978, 1992.

TAKEDA, R.; IKEDA, T.; TSUDA, F.; ABE, H.; HASHIGUCHI, H.; ISHIDA, Y.; NISHIMORI, Y. Unilateral lesions of mesostriatal dopaminergic pathway alters the withdrawal response of the rat hindpaw to mechanical stimulation. Neuroscience Research, v. 52, p. 31–36, 2005.

THOENEN, H.; TRANZER, J.P. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hidroxydopamine. Naunyn Schmiedebergs Arch Experimental Pathology Pharmakol, vol.261, p.271-288, 1968.

UNGERSTEDT, U.; ARBUTHNOTT, G. W. Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. Brain Research, v. 24, p. 485-493, 1970.

UNGESTEDT, U. 6-hidroxydopamine induced degeneration of central monoamine neurons. European Journal of Pharmacology, vol. 5, p. 107-110, 1968.

VANDERHAEGHEN, J.J.; PERIER, O.; STERNON, J. E. Pathological findings in idiopathic orthostatic hypotension. Its relationship with Parkinson´s disease. Archiology Neurology. vol. 22, p.207-214, 1970.

WICHMANN, T.; DeLONG, M.R. Neurotransmitters and desorders of the basal ganglia. In: SIEGEL, G.J. Basic Neurochemistry: Molecular, Celular and Medica aspect. 7th edition, Elsevier Academic press, Canadá. 2006.

WINKLER, C.; BENTLAGE, C.; NIKKHAH, G.; SAMILI, M.; BJÖRKLUND, A. Intranigral transplants of GABA-rich striatal tissue induce behavioral recovery in the rat Parkinson model and promote the effects obtained by intrastriatal dopaminergic transplants. Experimental Neurology, v.155, p.165–186, 1999.

YOUDIM, M. B. H.; RIEDERER, P. Understanding Parkinson´s Disease. Scientific American,, v. 276, p.52–59, 1997.

YUAN, H.; SARRE, S.; EBINGER, G.; MICHOTTE, Y. Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. Journal of Neuroscience Methods. V. 144, p. 35–45, 2005.

ZETTERSTROM, T., HERRERA-MARSCHITZ, M., UNGERSTEDT, U. Simultaneous measurement of dopamine release and rotational behaviour in 6-hydroxydopamine denervated rats using intracerebral dialysis. Brain Research. V. 376, p.1– 7, 1986.

ZIGMOND, M.J.; ACHESON, A.L.; STACHOWIAK, M.K.; STRICKER, E.M. Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonism. Archiology Neurology, v. 41, p. 856–61, 1984.