UNIVERSITAT DE BARCELONA

Estudi de la degeneració transneuronal en models de malalties que afecten als ganglis basals

Anna-Maria Canudas Teixidó

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA

Departament de Farmacologia i Química Terapèutica

Programa de Doctorat en Farmacologia. Bienni 1996-1998

ESTUDI DE LA DEGENERACIÓ TRANSNEURONAL EN MODELS DE MALALTIES QUE AFECTEN ALS GANGLIS BASALS

Tesi doctoral presentada per Anna-Maria Canudas Teixidó,
per optar al títol de Doctor en Farmàcia, sota la direcció dels Drs.
Jordi Alberch Vié, Jordi Camarasa García i Mercè Pallàs Lliberia,
essent tutor el Dr. Jordi Camarasa García.

Barcelona, Juny del 2001

L'estudi de les malalties neurodegeneratives representa un dels principals objetius de la farmacologia actual degut a l'augment de la seva incidència en les últimes dècades. El fet que l'evolució d'aquestes malalties es caracteritzi per una pèrdua progressiva de la qualitat de vida del malalt, el que comporta associat un deteriorament de l'entorn familiar, implica que l'estudi de l'etiologia, diagnòstic i noves teràpies d'aquest tipus de malaties neurològiques sigui un objetiu rellevant en la nostra societat.

Una de las prioritats farmacològiques és el diagnòstic precoç de les malalties neurodegeneratives per permetre així, el més ràpid possible, la instauració del tractament fins avui únicament paliatiu. Un dels punts importants doncs, per arribar a aquest objectiu, és aprofundir en el coneixement de la fisiopatologia de les malaties neurodegeneratives estudiant els mecanismes de neuroprotecció.

La disponibilitat de determinats models animals per malalties humanes facilita molt la investigació, ja que permet una major accessibilitat als teixits, una capacitat de control dels agents externs i la possibilitat de provar noves teràpies, que no són possibles quan l'estudi s'efectua en humans. Els models més fidels reflexen la malaltia apareguda espontàniament i permeten obtenir conclusions vàlides; però, tot i així és important comparar rigurosament el model amb els trets característics de la malaltia en humans.

La malaltia de Parkinson i la corea de Huntington s'inclouen entre les malalties neurodegeneratives. Aquests síndromes són uns trastorns crònics i progressius del sistema nervios central, caracterizats per alteracions en el moviment, i són *in extremis*, incapacitants.

Els ganglis basals són els nuclis que es troben principalment implicats en aquestes malalties neurodegeneratives associades amb activitats motores anormals. Aquests nuclis es troben interconnectats, el que provoca que una lesió inicial en un nucli en concret, pugui també ocasionar degeneració neuronal en zones distants a aquest punt incial de lesió el que provocarà una amplificació de la patologia. Aquest trastorn és conegut com a degeneració transneuronal.

Una lesió en el sistema nerviós va acompanyada d'un seguit de respostes endògenes defensives i regeneratives, entre aquestes respostes una de les més importants és la reacció glial, ja sigui microglial o astroglial. La glia forma part de la població normal del sistema nerviós, i en certes situacions pot patir diferents canvis morfològics,

neuroquímics i fenotípics. Si aquesta transformació glial té un paper neuroprotector o neurotòxic continua essent un tema de discussió. S'ha postulat sobre la modificació d'aquesta gliosis com a estratègia a fi d'establir un ambient favorable per la reparació neuronal. Així doncs, els estudis encarats a aprofundir en els processos glials ajudarà a desenmascarar alguns punts encara per conèixer de la reactivitat glial que té lloc en els processos degeneratius del sistema nervió central.

A més d'aquesta reacció glial també s'han observat canvis en els nivells de factors tròfics, dins aquests trobem les neurotrofines. En els últims anys s'han realitzat molts estudis tan *in vivo* com *in vitro* on han mostrat un efecte promotor de la supervivència i neuroprotector de les neurotrofines sobre les poblacions neuronals dels ganglis basals. Per això s'ha proposat a les neurotrofines com a possibles candidates a agents terapèutics en les malalties. Alguns autors han postulat que l'origen de les malalties degeneratives ve donat per una alteració en els nivells endògens de factors tròfics. Això ha estimulat la recerca sobre el coneixement de la implicació i regulació d'aquests factors en trastorns neurodegeneratius. Induint la malaltia experimental podrem aproximar-nos a la degeneració neuronal que té lloc en els ganglis basals i evaluar els canvis endògens d'aquests factors que tenen lloc, així com el seu efecte neuroprotector quan s'administren de forma exògena.

1.-ELS GANGLIS BASALS

Els **ganglis basals** són un conjunt de nuclis subcorticals implicats en una gran varietat de processos, entre els que s'inclouen funcions motores, associatives, cognitives i mnemotècnies. Els nuclis dels ganglis basals són: 1) El nucli estriat (el nucli estriat dorsal format pel nucli caudat i el nucli putamen, i el nucli estriat ventral que inclou el nucli accumbens), 2) El *globus pallidus*, que conté un segment intern i un extern, 3) El nucli subtalàmic i 4) La substància negra, que es divideix anatòmicament en *pars reticulata* i *pars compacta* (Parent i col., 1996, Bolam i col., 2000).

L'entrada més important de informació en els ganglis basals prové de l'escorça cerebral. El principal punt d'entrada de la informació cortical als ganglis basals és el

nucli estriat, tot i que també existeixen projeccions de l'escorça cerebral al nucli subtalàmic. El nucli estriat la processa i la integra amb els altres senyals que arriben també al nucli estriat des del rafe i el *locus coeruleus*, des de varis nuclis talàmics, i des de la *pars compacta* de la substància negra. Un cop processada, la informació és transmesa a les estructures de sortida, el segment intern del *globus pallidus* i la *pars reticulata* de la substància negra. És a través d'aquestes estructures que la informació estriatal pot tornar cap a l'escorça cerebral via el talem, tancant així el circuit conegut com cortico-estriat-tàlem-cortical (Parent, 1995, Bolam i col., 2000).

Els ganglis basals són, doncs, un conjunt complex de nuclis, altament interconnectats, que han sigut objecte d'estudi durant moltes dècades, principalment degut a la seva clara implicació en les malalties neurodegeneratives associades amb activitats motores anormals. Les alteracions en la connectivitat del complex estriatal i dels seus aferents comporten trastorns de caràcter motor com la acinèsia o dificultat per iniciar els moviments, moviments lents o bradicinèsia i moviments involuntaris anormals o discinèsia, associades a alteracions del to muscular, hiper o hipotonia. Entre les malalties neurodegeneratives que afecten als ganglis basals trobem la malaltia de Parkinson i la corea de Huntington.

1.1.-EL NUCLI ESTRIAT

El nucli estriat està compost per neurones de projecció i circuits locals, interneurones, però es diferencia d'altres àrees cerebrals en que les neurones de projecció superen en gran número a les interneurones (Graveland, 1985).

1.1.1.-Tipus de neurones del nucli estriat

1.1.1.a.-Les neurones de projecció del nucli estriat tenen un cos cel·lular mitjà (12-20 μm de diàmetre) des del que radien 4-5 dendrites primàries que en la regió més propera al soma són primes i augmenten el seu gruix a mida que incrementa el seu número d'espines (Wilson i Groves, 1980). Aquests neurones s'han anomenat neurones

mitjanes espinoses. Els seus axons surten del soma o d'una dendrita pròxima al tronc i emet varis col·laterals abans d'allunyar-se del cos cel·lular. La majoria d'aquestes utilitzen com a neurotransmissor principal l'àcid γ-aminobutíric (GABA) (Ribak, 1979; Oertel, 1984), però també coexpressen pèptids neuroactius, com la substància P, encefalines, dinorfina i neurotensina. Aquests pèptids es troben en diferents tipus de neurones i la seva colocalització amb el GABA té una correlació amb l'òrgan diana al que projecten. Les neurones estriatals que projecten monosinàpticament a la part interna del *globus pallidus* i a la substància negra *pars reticulata*, coneguda com la via directa conté substància P i dinorfina, mentre que les neurones que projecten al segment extern del *globus pallidus* i al nucli subtalàmic, conegut com la via indirecta, expressen encefalines (Gerfen, 1992). Comparat amb l'acció inhibitòria molt bé caracteritzada del GABA, el paper d'aquests pèptids neuroactius encara resta per determinar.

1.1.1.b.-Existeixen una gran varietat d'**interneurones** en el nucli estriat que comparteixen una característica comuna, l'absència o rara presència d'espines en les dendrites, contrastant amb les dendrites altament espinoses de les neurones de projecció. Aquests neurones estriatals es poden agrupar en dues categories: a) les neurones grans sense espines dendrítiques que utilitzen acetilcolina com a neurotransmissor (Bolam i col., 1984); i b) les neurones mitjanes sense espines que normalment es divideixen en dues categories, en funció del contingut en neurotransmissor. El primer tipus presenta immunoreactivitat pel GABA i l'enzim àcid glutàmic descarboxilasa (GAD), i també conté parvalbúmina (Cowan i col., 1990; Kawaguchi i col., 1993; Kita i col., 1990). El segon tipus de neurones conté somatostatina (DiFiglia i Aronin, 1982a; Takagi i col., 1983) i neuropèptid Y (Smith i Parent, 1986) però no contenen mRNA per GAD (Chesselet i Robbins, 1989). Aquestes neurones també contenen l'enzim nicotinamida adenina dinucleotid fosfat (NADPH)-diaforasa. Més recentment s'ha descrit un tercer tipus de neurones mitjanes que conte calretinina (Bennett i Bolam, 1993; Cicchetti i col., 1998).

1.1.2.-Estructura del nucli estriat

El coneixement de la diversitat neuroquímica de cada un dels diferents tipus cel·lulars ha permès trencar amb la idea de que el nucli estriat és un nucli homogeni sense una estructura en làmines o compartiments, obtinguda mitjançant les tècniques de impregnacions argèntiques.

S'ha demostrat que la citoarquitectura del nucli estriat és heterogènia i que presenta dues regions ben diferenciades, proposades per Graybiel i Ragsdale al 1978:
a.-Els estriosomes que són definits per regions riques en receptors μ per a opiàcis (Herkenham i Pert, 1981) i pobres en marcatge per l'acetilcolinesterasa (Graybiel i Ragsdale, 1978); i **b.- La matriu** que és la regió complementària als estriosomes, és rica en marcatge per l'acetilcolinesterasa (Graybiel i Ragsdale, 1978), la calbindina (proteïna queladora de calci) i en fibres immunoreactives per la somatostatina (Gerfen, 1985; Gerfen i col., 1985).

Aquests compartiments han estat ben definits en la rata i s'ha observat una organització similar en els primats i en el gat. La distribució immunohistoquímica dels pèptids: encefalines i substància P (Becksteat i Kersey, 1985; Gerfen, 1984), algunes de les aferències (Malach i Graybiel, 1986) i de les eferències (Giménez-Amaya i Graybiel, 1990) del nucli estriat, presenten patrons no sempre coincidents amb els compartiments definits com estriosomes o matriu (per revisar el tema: Gerfen, 1992).

1.1.3.-Compartimentació funcional del nucli estriat.

Tot i que els marcadors neuroquímics han servit per definir els compartiments del nucli estriat, sembla ser que aquesta organització també està relacionada amb diferents poblacions de neurones mitjanes amb espines que presenten diferents aferències i eferències (Gerfen, 1984; 1985; Gerfen i col., 1985).

a.- Eferències: En rates, el marcatge retrògrad va mostrà que tant les neurones dels estriosomes com les de la matriu projecten a la substància negra, però a diferents zones. Les neurones dels estriosomes contacten amb neurones dopaminèrgiques de la

pars compacta i de la *pars reticulata* de la substància negra mentre que les neurones de la matriu contacten amb neurones GABAèrgiques de *la pars reticulata* de la substància negra (Gerfen 1984; 1985; Gerfen i col., 1985). La immunreactivitat per la calbindina, la qual marca neurones de projecció de la matriu, va confirmà, per la seva distribució específica en zones no-dopaminèrgiques de la *pars reticulata* de la substància negra, l'organització estriosoma-matriu de la projecció estriato-nigral (Gerfen i col., 1985).

b.-Aferències: les projeccions que arriben al nucli estriat a partir de neurones dopaminèrgiques de l'àrea tegmental ventral, de la substància negra i de l'àrea retrorubral estan també compartimentades (Gerfen, 1992). Els terminals que arriben a la matriu provenen de neurones dopaminèrgiques situades de manera contínua en l'àrea tegmental ventral, de la banda dorsal de la substància negra *pars compacta* i de l'àrea retrorubral. Els terminals dirigits als estriosomes provenen de neurones dopaminèrgiques de la *pars compacta* de la substància negra i de neurones dopaminèrgiques que s'agrupen en illots, en la *pars reticulata* de la substància negra. Les neurones dopaminèrgiques que projecten a la matriu contenen calbindina mentre que les que ho fan als estriosomes, no (Gerfen, 1992). Les connexions cortico-estriatals, també mostren patrons diferents, la matriu rep aferències corticals de l'escorça parieto-temporo-occipital associativa, de l'escorça sensorimotora i parts de l'escorça frontal lateral (Ragsdale i Graybiel, 1981; Ragsdale i Graybiel, 1990) mentre que els estriosomes reben aferències corticals del cortex prefrontal i limbic (Gerfen, 1984).

Aquests dos compartiments estriatals estan funcionalment connectats mitjançant interneurones que contenen somatostatina o neuropèptid Y (Chesselet i Graybiel, 1986; Gerfen, 1992).

Estudis realitzats en rates lesionades amb 6-hidroxidopamina, substància que destrueix les neurones dopaminèrgiques, van suggerir que les aferències estriatals podien estar relacionades amb diferents poblacions de receptors de dopamina (Herrera-Marschidtz i Ungerstedt, 1984). Posteriorment, estudis de hibridació *in situ* han permès concloure que les neurones estriatals que projecten al *globus pallidus* intern i a la *pars reticulada* de la substància negra expressen principalment receptors D1, mentre que les neurones que projecten al *globus pallidus* extern expressen principalment receptors D2 (Gerfen, 1992; Reiner i Anderson, 1990)

1.2.-LA SUBSTÀNCIA NEGRA

1.2.1.-Divisió anatòmica i característiques fenotípiques de les poblacions de la substància negra.

La substància negra es pot dividir anatòmicament en dues parts, la ***pars compacta*** i la ***pars reticulata***. La *pars compacta* és més rica en cèl·lules que la *pars reticulata*, mentre que l'última, com el seu nom indica, és més rica en arboritzacions dendrítiques.

Les neurones residents en la substància negra contenen diferents poblacions fenotípiques. Les neurones de la substància negra *pars compacta* són principalment dopaminèrgiques i s'utilitza com a marcador la tirosina hidroxilasa (TH), enzim de la síntesi de la dopamina (Dahlstrom i Fuxe, 1964). Una gran subpoblació d'aquestes neurones nigrals dopaminèrgiques també contenen el neuropèptid colecistocinina (CCK) (Fallon i col., 1983; Fallon i Seroogy, 1985; Seroogy i col., 1989). Entre un 50 i un 75% de les neurones TH positives de la substància negra *pars compacta* contenen la proteïna queladora del calci, calretinina, i un terç d'aquestes també contenen calbindina (Gerfen i col., 1985; Resibois i Rogers, 1992; Rogers, 1992; Isaacs i Jacobowitz, 1994; Liang i col., 1996; McRitchie i col., 1998). La CCK col·localitza amb la subpoblació calbindina de les neurones dopaminèrgiques de la substància negra *pars compacta* (German i Liang, 1993), i sembla ser que es distribueix en les calretinines restants també, ja que la CCK es presenta en una població més gran que les calbindines. Les neurones de la substància negra *pars reticulata* són principalment GABAèrgiques (Oertel i col., 1982), algunes també contenen parvalbúmina (Gerfen i col., 1985; Celio, 1990; Hontanilla i col., 1997; McRitchie i col., 1998). A la part ventral de la substància negra *pars reticulata* s'hi localitzen algunes neurones dopaminèrgiques (Faull i Mehler, 1978; Becksteat i col., 1979; Deutch i col., 1986).

1.2.2.- Les connexions de la substància negra.

Les **connexions aferents** de la substància negra surten de diferents grups cel·lulars, però quantitativament l'entrada més important prové del nucli estriat. Tot i que la majoria de les fibres estriatonigrals acaben en la *pars reticulata*, les cèl·lules de la *pars compacta* també poden estar influenciades per aquesta via perquè les seves llargues dendrites es poden extendre a la *pars reticulata* (fig.1). Les fibres estriatonigrals tenen com a neurotransmissor principal el GABA, que exerceix una acció inhibitòria sobre les cèl·lules de la substància negra (fig.3). Altres aferents de la substància negra provenen del *globus pallidus* (GABA), del nucli subtalàmic (glutamat), del *locus coeruleus* (noradrenalina), i dels nuclis del rafe (serotonina).

Les **connexions eferents** de la substància negra passen principalment pel nucli estriat i pel nucli talàmic, i amb menys densitat als grups cel·lulars de la formació reticular del colícul superior. Les neurones dopaminèrgiques nigroestriatals es localitzen en la *pars compacta* de la substància negra, mentre les neurones nigrotalàmiques GABAèrgiques estan localitzades en primer lloc en la *pars reticulata* de la substància negra. Les neurones nigrals que envien axons al nucli colícul també es troben en parts separades de la substància negra. A més, els aferents que provenen de diferents parts del nucli estriat (com els nuclis caudat o putamen) acaben en punts diferents. La substància negra sembla ser que consisteix en vàries parts que difereixen en funcionalitat, i que es diferencien entre elles amb els aferents i els grups cel·lulars on exerceixen la seva acció.

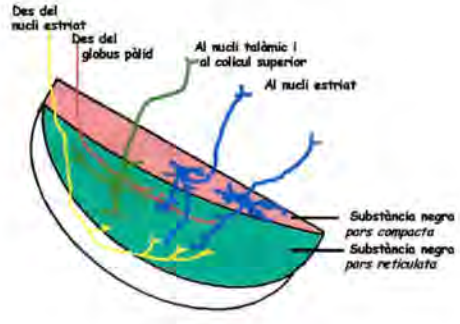

Fig.1. La substància negra.

1.3.-L'ESCORÇA CEREBRAL

1.3.1.-Citoarquitectura del neocortex

Totes les parts del neocortex comparteixen una estructura bàsica única, amb les neurones organitzades en sis capes orientades paral·lelament a la superfície de l'escorça (fig.2). Una altra característica general és l'organització de les neurones en columnes orientades perpendicularment a la superfície cortical. Els dos tipus d'organització estan en relació amb l'especialització de la seva funció. En la figura 2 es pot observar les característiques de les capes, aquest patró laminar ve donat per la localització de les neurones de similar forma i mida. En les diferents capes es troben diferents densitats dels cossos cel·lulars.

Uns dos terços de les neurones corticals són les **cèl·lules piramidals**, que com el seu nom indica els seus cossos tenen forma cònica. Aquestes cèl·lules tenen un axó llarg que surt de la base de la piràmide i una dendrita apical que s'exté per la superfície cortical, per capes més superficials que la capa on es localitza el pericarion i les dendrites basals. Una característica primordial d'aquests cèl·lules és que la seva superfície conté un gran nombre d'espines dendrítiques que augmenten considerablement la seva superficie. La resta de neurones corticals constitueixen un grup heterogeni el qual les seves neurones tenen en comú que el seu pericarion no té forma piramidal, i són conegudes com neurones **no piramidals**. La seva forma i mida varia però sembla ser que la majoria són interneurones. L'exemple més representatiu d'interneurona és la cèl·lula granular estrellada de petit tamany i amb un axó curt que es ramifica profusament. Altres interneurones són les neurones horitzontals de Cajal, i les cèl·lules de Martinotti, que tenen un axó ascendent.

L'estructura en capes està formada per la **capa I** o **capa molecular,** que és la capa més superficial, molt rica en fibres, amb cèl·lules horitzontals de Cajal-Retzius durant el desenvolupament i poques interneurones més. A part d'axons, conté les dendrites apicals de les neurones piramidals de les capes més profundes. La **capa II** o la **capa granular externa**, conté cèl·lules granulars estrellades. La **capa III** o **capa**

piramidal externa es pot reconèixer pel seu contingut en cèl·lules piramidals de tamany petit i mitjà; la **capa IV**, la **capa granular interna**, té una construcció similar a la capa II. La **capa V**, la **capa piramidal interna**, conté cèl·lules piramidals mitjanes i grans. La **capa VI**, la **capa multiforme**, conté cèl·lules de diversa morfologia, tals com les cèl·lules fusiformes i les neurones Martinotti.

Es pot dir que la capa II i la IV són principalment receptores, mentre que les capes III i V són principalment eferents, i envien els seus axons fora de la zona de l'escorça on estan localitzades. Les cèl·lules piramidals de la capa V envien els seus axons principalment a nuclis subcorticales. Les cèl·lules piramidals de la capa III envien els seus axons principalment a altres àrees de l'escorça. La capa VI també presenta molts eferències i envia moltes connexions al nucli talàmic.

Les capes corticals també es diferencien respecte amb l'origen de les seves aferències extrínsiques. Existeix un cert grau d'especialització entre les capes en quan al tipus de informació que processen. En les zones motores tindria un predomini la capa V mentre que en les zones sensitives predominaria la capa IV.

Com ja s'ha comentat, l'escorça cerebral s'organitza en columnes cilíndriques, semblants a petits barrils, els diàmetres dels quals es corresponen amb les ramificacions de les fibres aferents específiques. D'aquesta forma i en un espai petit de no més de 500 μm de diàmetre, les aferències específiques finalitzen sobre les interneurones corticals, els axons de les quals solen convergir sobre les cèl·lules de projecció de l'interior de la columna.

Tot i que totes les parts del neocortex tenen sis capes, el gruix i l'estructura de vàries capes pot canviar d'una àrea a l'altra. Aquestes diferències citoarquitectòniques difereixen unes de les altres formant una subdivisió de tota l'escorça en àrees citoarquitectòniques, com van realitzar en Brodmann i altres (Brodmann, 1909). Tot i que al principi les diferents àrees sols es van definir en base a la seva mida, forma i organització dels seus cossos cel·lulars, s'ha provat la importància d'aquesta divisió degut a que poden diferir també en funció. Alguns punts d'unió entre les diferents àrees citoarquitectòniques es poden diferenciar clarament mentre que altres són realment imperceptibles.

Fig.2. Organització cel·lular en capes del neocortex

1.4.-CIRCUIT RECURRENT SUBSTÀNCIA NEGRA-NUCLI ESTRIAT-ESCORÇA CEREBRAL.

El coneixement de l'organització anatòmica, neuroquímica i fisiològica així com dades *post-mortem*, van permetre formular un model unificador de l'organització funcional dels ganglis basals (Albin i col., 1989). Aquest model, que ha sigut extès i el·laborat per varis grups, es basa en una via directa i una via indirecta del flux de

informació de l'escorça cerebral cap als ganglis basals (fig.3).

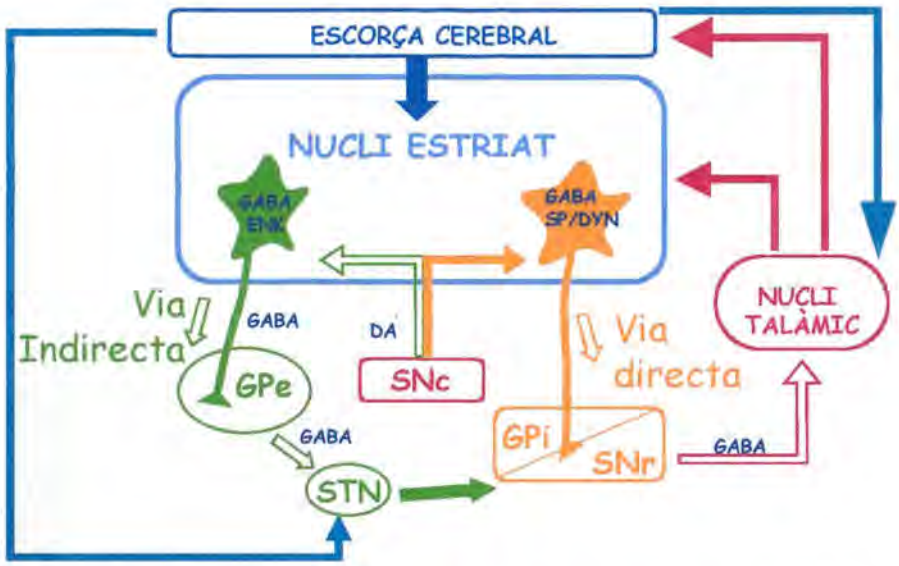

Fig.3.-. Circuits dels ganglis basals, les projeccions inhibitòries es mostren com les fletxes buides, les projeccions excitadores es mostren com fletxes plenes. DA, dopamina; enk, encefalinas; Gpi *Globus pallidus* interno, GPe *Globus pallidus* extern Subst P substància P, SNc Substància negra *pars compacta*, SNr substància negra *pars reticulata*.

D'acord amb aquest model, la informació cortical que arriba al nucli estriat és processada i transmesa a les estructures de sortida dels ganglis basals, el *globus pallidus* intern i la substància negra *pars reticulata* via dues rutes, amb una projecció inhibitòria directa des del nucli estriat al *globus pallidus* intern/substància negra *pars reticulata* o amb una ruta indirecta que involucra una projecció inhibitòria des del nucli estriat al *globus pallidus* extern, una projecció inhibitòria des del *globus pallidus* extern al nucli subtalàmic i una projecció excitadora des del nucli subtalàmic al *globus pallidus* intern/substància negra *pars reticulata*. La informació és transmesa altre cop cap a l'escorça cerebral via el nucli talàmic. La ruta directa i la ruta indirecta sorgeixen de diferents poblacions de neurones espinoses estriatals que contenen diferents pèptids i preferentment expressen diferents subclasses de receptors dopaminèrgics. Les neurones

dopaminèrgiques de la substància negra *pars compacta* exerceixen un efecte net excitador en les neurones espinoses originant la ruta directa per l'activació dels receptors D_1, mentre elles exerceixen un efecte inhibidor net en les neurones espinoses donant origen a la ruta indirecta per l'activació dels receptors D_2.

2.-MALALTIES QUE AFECTAN ALS GANGLIS BASALS

2.1.-LA MALALTIA DE PARKINSON

La malaltia de Parkinson és una malaltia discapacitant, els pacients que pateixen aquesta patologia desenvolupen uns símptomes molt característics com la rigidesa muscular, el tremolor estàtic i un enlentiment del moviment (bradicinèsia). A més pot presentar-se una falta d'expressió facial, una postura flexionada, una pèrdua en l'agilitat dels dits, pell grassa i dificultat en la deglució.

Aquesta malaltia apareix a qualsevol part del món, en tots el grups étnics, i es presenta en ambdós sexes de forma similar, amb una discreta predominància en els homes. La prevalència augmenta exponencialment entre els 65 i els 90 anys (Morens i col., 1996). Hi ha una propensió universal per el desenvolupament de la malatia, però els factors locals de l'entorn, poden jugar un paper en la seva presentació. Existeixen varis agents tòxics, capaços de provocar la malaltia de Parkinson simptomática, com els neurolèptics, els antagonistes del calci, o l'1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP), substància que a més de provocar simptomes parkinsonians, indueix mort cel·lular selectivament en la substància negra . S'ha associat l'entorn rural amb un elevat risc de patir la malatia, degut a l'exposició a pesticides o herbicides (Semchuck i col., 1998). Un factor associat consistentment amb un risc reduït de patir la malatia de Parkinson és l'hàbit tabàquic (Tzourio i col., 1997). Existeix una evidència creixent que els factors genètics, juguen un paper important en la malatia. Alguns estudis epidemiològics, han trobat que apart de l'edat, la historia familiar de la malaltia de

Parkinson és un predictor de risc de patir la malatia, tot i així el paper en aquests casos d'una exposició ambiental, ha de ser tingut en consideració.

Recentment, s'han indentificat dues mutacions diferents, en el gen de la α-sinucleïna, localitzat en el cromosoma 4q. Una mutació es va trobar en una extensa família italiana i en tres gregues, i l'altra, en una família d'orígen germànic. Aquesta proteïna, de funció desconeguda, s'expressa principalment en els terminals presinàptics del cervell. Més recentment, s'han descrit mutacions en el braç llarg del cromosoma 6 (Polymeropoulos i col., 1997; i Bandman i col., 1998). El producte proteic, denominat *parkin*, és homòleg a la ubiquitina, família de proteïnes implicades en la patogènesi de diverses malalties neurodegeneratives (Kitada i col., 1998). La majoria de pacients no tenen una clara història familiar de malaltia autosòmica dominant, probablement perquè els gens causants tenen una penetrància baixa, o perquè la causa de la malatia és multifactorial (una combinació de la predisposició genètica i l'exposició ambiental).

La principal causa d'aquesta malaltia és la progressiva pèrdua de neurones dopaminèrgiques en la *pars compacta* de la substància negra, acompanyada per l'aparició de inclusions intracitoplasmàtiques conegudes com els cossos de Lewy.

En la malaltia de Parkinson, degut a una lesió destructiva de la substància negra, no es forma dopamina, el que produeix un dèficit d'aquesta en la substància negra i en el nucli estriat (per degeneració de les fibres nigroestriades). La manca de dopamina a nivell del nucli estriat anul·la l'acció inhibidora d'aquest neurotransmissor, el que dóna origen a l'augment del to muscular i als trastorns del moviment observats en el parkinsonisme.

Però a més, l'acetilcolina també té un paper en la fisiologia i fisiopatologia del nucli estriat, ja que aquest neurotransmissor posseeix accions estimulants sobre el nucli putamen i el nucli caudat, podent-se'l considerar com un antagonista de la dopamina; és possible que el dèficit de dopamina en el parkinsonisme porti a un predomini de l'acció de les fibres colinèrgiques i a l'estimulació conseqüent del nucli estriat. Així, es pot considerar al parkinsonisme com originat per un desequilibri entre els sistemes dopaminèrgic i colinèrgic a nivell dels ganglis basals, produït per una deficiència primària de dopamina.

En els anys 60 es va inicià un tractament substitutiu per aquesta malaltia amb la L-3,4-dihidroxifenilalanina (L-Dopa), el precursor de la dopamina. En molts pacients la L-Dopa pal·lia un gran nombre de símptomes, especialment la tremolor i la rigidesa. Desafortunadament, el tractament crònic amb L-Dopa comporta la desaparició dels efectes pal·liatius produint unes fluctuacions diàries conegudes com efecte de "on-off". A més, després d'un tractament crònic amb L-Dopa es poden desenvolupar unes discinèsies que poden arribar a ser molt severes i causar discapacitat. Al final, la neurodegeneració progressa i la L-Dopa perd la seva efectivitat.

2.1.1.-Models experimentals en l'estudi de la malaltia de Parkinson

La destrucció unilateral de neurones nigrals en rosegadors i primats resulta en un desequilibri estriatal de la dopamina presinàptica i en una supersensibilitat del receptor per la dopamina post-sinàptica en el cantó denervat.

2.1.1.a.-Mecànic:

Un model tradicional és l'axotomia mecànica dels aferents nigrals utilizant un bisturí especial. La transecció de la via nigroestriada resulta en una degeneració retrògrada dels cossos cel·lulars dopaminèrgics del mesencèfal. (Knusel i col., 1992; Beck i col., 1995; Alexi i Hefti, 1996; Tseng i col., 1997; Hagg, 1998)

El grau de lesió depèn de la proximitat del tall als cossos cel·lulars de la substància negra i l'extensió amb què es talla la via nigroestriada. La transecció nigroestriada provoca un dany mecànic a les neurones, una pèrdua de transport intracel·lular a través dels axons i un estrés oxidatiu (Leah i col., 1993; Venero i col.; 1997; per revisió Koliatsos i Price, 1996). Aquest tipus d'axotomia sembla ser que causa una mort de les cèl·lules nigrals principalment de naturalesa necròtica (Venero i col.,1997)

2.1.1.b.-Neuroquímics

2.1.1.b.1.-6-hidroxi-dopamina:

Generalment aquesta neurotoxina és administrada unilateralment a la via nigroestriada, al nucli estriat, o a vegades directament a la substància negra resultant en la seva captació selectiva per les neurones dopaminèrgiques (i altres catecolaminèrgiques pròximes) on produeix un estrés oxidatiu i finalment una degeneració cel·lular (Cohen i Heikkila, 1974). Normalment s'administren també inhibidors de la captació de noradrenalina a fi de prevenir la lesió del *locus coeruleus*. L'extensió de la degeneració de les cèl·lules nigrals induïda per la 6-hidroxidopamina és dosi-depenent i es pot aconseguir una completa desaparició de l'activitat tirosina hidroxilasa (Sauer i Oertel, 1994).

Aquesta lesió produeix un comportament rotacional típic que es pot quantificar i correlacionar amb la funció nigroestriatal (Ungerstedt, 1971a, 1971b). Per aquest estudi rotacional s'utilizen comunment dos fàrmacs: l'amfetamina i l'apomorfina. L'amfetamina actua en l'alliberació presinàptica de dopamina i indueix rotacions ipsilaterals en animals lesionats unilateralment perquè s'allibera més dopamina en l'hemisferi intacte que en el lesionat, resultant en una sobreactivació del no lesionat produint que l'animal mogui més el cantó no lesionat i provocant que l'animal camini en cercles cap al cantó lesionat. L'apomorfina és un agonista del receptor dopaminèrgic a nivell postsinàptic que indueix rotacions contralaterals a la lesió ja que ara, el cantó lesionat està sobreactivat respecte l'intacte, degut a que la lesió indueix una supersensibilitat dels receptors dopaminèrgics.

2.1.1.b.2.- MPTP:

Al 1983 Langston va descriure a tres joves drogoaddictes que, aparentment, patien la malaltia de Parkinson. Va verificar que el derivat piridínic MPTP, trobat en

l'heroïna utilitzada pels drogoaddictes era la responsable del ràpid desenvolupament d'una síndrome clínicament indistingible de la malaltia de Parkinson avançada (Davis i col., 1997). Va relacionà els seus descobriments amb un cas similar exposat anteriorment on s'observà, *post mortem*, una destrucció de la *pars compacta* de la substància negra del jove afectat. Estudis en animals, mostraren que les lesions del cervell, els canvis bioquímics i les anormalitats motores produïdes per l'MPTP eren molt similars a les trobades en la malaltia de Parkinson espontània (Burns i col., 1983).

Després de l'administració de l'MPTP en primats, aquesta droga desapareix de tots els teixits excepte de l'ull (Langston i col., 1984a) i produeix metabòlits bàsics, àcids o neutres, no detectables en extractes orgànics o en teixits biològics. Durant una hora es poden detectar quantitats elevades del ió 1-metil-4-fenilpiridini o MPP^+, altament soluble en aigua, en teixits perifèrics i en el sistema nerviós central (Langston i col., 1984a; Markey i col., 1984). Tot i que la conversió de piridines parcialment reduïdes a compostos piridini s'ha proposat com un nou sistema de distribució cap al cervell, aquesta oxidació de quatre electrons no és una via metabòlica comunment descrita en el sistema nerviós central. En contrast amb la ràpida desaparició de l'MPTP, l'MPP^+ és extremadament persistent en el sistema nerviós central de primats, amb una vida mitja de 48-100 h (Markey i col., 1984; Irwin i col., 1985). De fet, estudis en primats han mostrat que les concentracions de l'MPP^+ comencen a disminuir entre les 24 i 72 h després de l'administració, en la major part del cervell i en la resta del cos, però en la substància negra s'incrementen i es mantenen durant uns dies (Irwin i col., 1985).

La biotransformació de l'MPTP a MPP^+ es produeix per un procés d'oxidació en dos passos, amb la formació del ió 1-metil-4-fenil-2,3-dihidropiridini o $MPDP^+$ com intermedi (Chiba i col., 1985) (fig.4). Aquesta reacció està mediada per la monoamino oxidasa B (MAO B) (Chiba i col., 1984a) que en la substància negra *pars compacta* està localitzada específicament en l'astroglia.

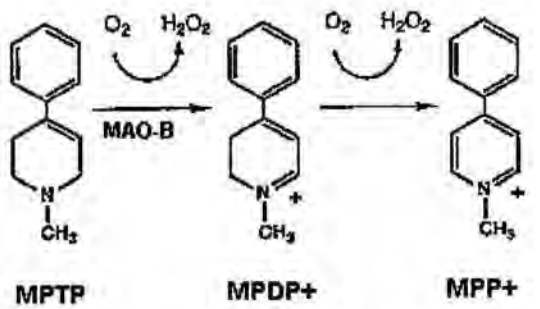

Fig.4. Generació enzimàtica de l'intermediari MPDP+, i la subseqüent formació espontània de l'MPP+.

Després de la seva formació, l'MPP^+ penetra en les neurones dopaminèrgiques a través del sistema de captació de dopamina (Javitch i col., 1985). En preparacions de sinaptosomes del nucli estriat, l'MPP^+ és captat amb la mateixa afinitat que la dopamina. Aquesta captació és reversible i competitiva amb la dopamina (Chiba i col., 1984b) i la seva inhibició pels diferents bloquejadors té el mateix ordre de potències per ambdues substàncies, dopamina i MPP^+, el que suggereix un lloc comú de transport (Javitch i col., 1985).

Per tant, l'MPP^+ s'acumula activament en les neurones dopaminèrgiques a través del sistema de captació de dopamina d'alta afinitat i produeix la seva acció neurotòxica mitjançant la inhibició de la respiració mitocondrial a nivell del complex I de la fosforilació oxidativa (Ramsay i col., 1991), i la subseqüent disminució d'ATP.

Amb l'MPTP es produeix una resposta astroglial derivada de l'alliberació de factors danyins des de les terminals dopaminèrgiques lesionades per l'MPP^+, les quals poden activar directament als astròcits residents (fig.5).

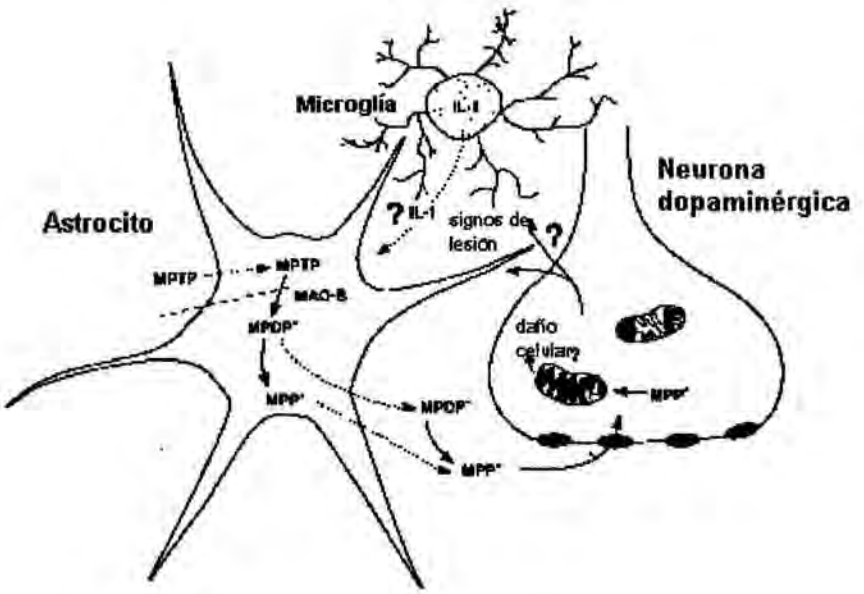

Fig.5. Model esquemàtic de la inducció de gliosis reactiva per l'MPTP.

A diferència dels primats, s'ha provat que la rata és refractària als efectes de l'MPTP. Alguns investigadors han trobat que no hi ha efectes sobre el contingut de dopamina en el teixit estriat després de l'administració sistèmica (Chiueh i col., 1984) i intranigral (Bradbury i col.,1986; Chiueh i col.,1984) de l'MPTP. En canvi, altres han descrit una disminució de la dopamina en el nucli estriat després de repetides injeccions subcutànies (Jarvis i col., 1985), d'una infusió contínua superior a les 24 h (Steranka i col., 1983), i després de la injecció en la substància negra de dosis molt altes d'MPTP.

S'han estudiat els efectes de l'MPP^+ en la rata. Degut a que aquest compost no travessa la barrera hematoencefàlica, ha de ser administrat directament en el sistema nerviós central. Quan s'injecta l'MPP^+ en el nucli estriat, en el feix del prosencèfal mitjà (Heikkila i col., 1985a) en la substància negra (Bradbury i col., 1986) de rates, es produeix una marcada disminució del contingut de dopamina en l'estriat. Injeccions bilaterals d'MPP^+ produeixen també una síndrome funcional que consisteix en acinèsia i rigidesa, per la qual cosa aquest metabòlit de l'MPTP és una valiosa neurotoxina experimental (Bradbury i col., 1986).

2.2.-LA MALALTIA DE HUNTINGTON

La malaltia de Huntington és un desordre autosòmic caracteritzat per una disfunció motora progressiva i alteracions cognitives (Harper, 1992; Folstein, 1998). Els trastorns motors són la principal característica de la malaltia i ha portat a utilitzar el terme de Corea de Huntington (corea, del grec dansa). Aquests trastorns inclouen tant les funcions motores voluntàries com les involuntàries. Els moviments involuntaris són de tipus coreiformes, moviments ràpids i incontrolats de les cames i dels músculs distals, i moviments involuntaris dels músculs proximals al tronc. La coordinació dels moviments voluntaris es torna progressivament més difícil i finalment arriba a ser impossible. Els símptomes cognitius inclouen canvis en la personalitat, depressió i demència. El patró de progressió de la simptomatologia pot variar molt entre pacients. Aquesta malaltia té una aparició en la fase adulta, a una edat mitja de 40 anys i té una duració mitjana d'uns 16 anys. A més existeix una forma juvenil que normalment té uns símptomes més severs i una esperança de vida més curta.

La corea de Huntington es caracteritza per una atròfia del nucli caudat i del nucli putamen deguda a la degeneració de les neurones estriatals (Martin i Gusella, 1986). Anàlisis *post-mortem* han demostrat que existeix una gran pèrdua de les neurones mitjanes espinoses de projecció GABAèrgiques estriatals, mentre que les interneurones colinèrgiques (Augood i col., 1996) i NADPHd són relativament resistents a la degeneració (Dawbarn i col., 1985). Les primeres cèl·lules que degeneren són les neurones de projecció GABAèrgiques que contenen encefalines i que innerven el *globus pallidus* extern (Reiner i col., 1998). Mentre aquests cèl·lules es van perdent progressivament les neurones de projecció GABAèrgiques que contenen substància P i projecten al *globus pallidus* intern i a la substància negra *pars reticulata* comencen a degenerar. En els últims estadis les neurones GABAèrgiques que projecten a la substància negra *pars compacta* es perden.

La malaltia de Huntington està causada per un increment de repeticions d'un trinucleòtid inestable en un gen de funció desconeguda, tot i que recentment s'ha descrit un paper en el tràfic intracel·lular. Aquest gen va ser localitzat en el cromosoma 4 al 1993 (Gusella, 1993). El gen *huntingtin* també conegut com IT15 (*interesting transcript 15*) va ser identificat al 1993. En persones que no tenen la malaltia el gen conté entre 9 i

34 repeticions CAG en la regió afectada en pacients amb la malaltia, mentre que la presència de 37 o més repeticions CAG dóna lloc a la malaltia de Huntington (Ashizawa i col., 1994; Gusella, 1993). De totes formes, algunes persones amb 37-39 repeticions poden o no estar afectats. Existeix una correlació inversa entre el número de repeticions CAG i l'edat d'aparició de la malaltia (Trottier, 1994; Brandt i col., 1996). Quan més alt el número de repeticions més aviat apareixen els símptomes. S'han descrit altres factors que podrien influenciar en l'edat d'origen de la malaltia.

El gen amb l'expansió del trinucleòtid CAG codifica per una regió poliglutamina en la proteïna producte, la huntingtina. L'ARNm del huntingtin dels al·lels normals expandits es troben àmpliament distribuïts pels teixits humans i s'expressa en neurones i glia de vàries regions del cervell amb nivells normals d'expressió en el nucli estriat de pacients presimptomàtics i en les neurones estriatals supervivents de cervells *post-mortem* de pacients amb la malaltia (Li i col., 1993; Strong i col., 1993; Landwehrmeyer i col; 1995; Schmitt i col; 1995; Stine i col., 1995). Aquesta àmplia distribució també s'ha trobat en la proteïna huntingtina, tan normal com expandida (Gutekunst i col., 1995; Trottier i col., 1995; Wood i col., 1996). El cervell té els nivells elevats d'expressió, ja sigui de l'ARN transcrit com de la proteïna comparat amb els teixits no neuronals (Schmitt i col., 1995; Sharp i col., 1995; Wood i col, 1996).

La majoria de neurones de projecció estriatals són positives per la hungtintina mentre que molt poques interneurones semblen expresar-la, ja sigui en rata o en humans (Ferrante i col., 1997; Kosinski, 1997). Aquest patró d'expressió és similar al patró neuropatològic observat en la malaltia, on les neurones de projecció degeneren mentre les interneurones estan relativament preservades.

No existeix un tractament efectiu per aquesta malaltia, normalment els pacients reben fàrmacs simptomàtics per les corees o la rigidesa i freqüentment, també, per la depressió.

2.2.1.-Models animals per la malaltia de Huntington

2.2.1.a.-Excitotoxicitat

L'expressió excitotoxicitat es creà al 1969 per descriure l'efecte neurotòxic dels aminoàcids excitadors que destruïen les neurones en el punt on eren injectats (Olney, 1969). L'excitotoxicitat pot jugar un paper principal molt important en la patogènesi de la malaltia de Huntington. S'han identificat diferents classes del receptor del glutamat i es divideixen en receptors ionotròpics i receptors metabotròpics lligats a proteïna G. Els receptors ionotròpics es classifiquen en dues subclasses (Hollmann i Heinemann, 1994; Watkins i Evans, 1981):

1.-N-metil-D-aspartat (NMDA): com agonistes selectius es coneixen l'NMDA i l'àcid quinolínic entre d'altres.

2.-No NMDA: aquest a la vegada es divideix en dos subtipus:

2.a.-Alfa-amino-3-hidroxi-5-metil-4-isoxazolepropionat (AMPA): amb agonistes selectius l'AMPA i l'àcid kaínic.

2.b.-El receptor de l'àcid kaínic: amb agonista l'àcid kaínic.

(Doble, 1995; Lipton, 1994).

Quan s'activen els receptors NMDA es tornen permeables al Ca^{2+} i al Na^{2+}. Es pensa que els receptors metabotròpics incrementen l'entrada de Ca^{2+} mediada pel receptor NMDA mitjançant l'activació d'una proteïna quinasa C lligada a una fosfolipasa (Schoepp i Sacaan, 1994). Les primeres observacions que varen vincular l'excitotoxicitat a la patogènesi d'aquesta malaltia van aparèixer al 1976, quan s'observà que la injecció intraestriatal d'àcid kaínic, agonista del receptor kainat, podia produir lesions similars a les observades en la malaltia (Coyle i Schwarcz, 1976; McGeer i McGeer, 1978).

En el punt de injecció les neurones estan depleccionades mentre que les fibres aferents, els components no neuronals i les interneurones estriatals grans no es troben afectades. Això va suggerir el possible paper del glutamat en la patogènesi de la malaltia de Huntington. La injecció intraestriatal de l'àcid quinolínic, agonista del receptor NMDA, produeix una lesió similar a la neuropatologia de Huntington, afectant

selectivament a les neurones estriatals de projecció (Beal i col., 1986; 1989; Huang i col., 1995). A diferència de l'àcid kaínic, l'àcid quinolínic és un metabòlit endogen del cervell. La lesió que produeix l'àcid quinolínic no afecta a la interneurones estriatals que contenen la somatostatina, el neuropèptid Y (Beal i col., 1989) i la NADPHd (Koh i col., 1986). La possibilitat de que els receptors AMPA o kainat tinguin un paper en la malaltia de Huntington és menor degut a que les lesions experimentals amb l'àcid kaínic i l'AMPA afecten a tots els tipus cel·lulars incloent les neurones somatostatina/ neuropèptid Y/ NADPHd del nucli estriat (Beal i col., 1986; 1989). Després de la injecció en el nucli estriat de rata, el compartiment estriosomal no es veu afectat mentre que les neurones de la matriu moren. A més, es produeix una degeneració primària de les neurones mitjanes espinoses eferents que projecten des del nucli estriat al *globus pallidus* i a la substància negra (Beal i col., 1989). Aquests resultats junt amb l'observació de que els receptors NMDA estan depleccionats en el nucli estriat humà de la malaltia de Huntington (Young i col., 1988), suggereix que l'excitotoxicitat mediada pels receptors NMDA té un paper en la fisiopatologia de la malaltia de Huntington i que les lesions experimentals amb l'àcid quinolínic en animals serveix com a model per aquesta malaltia.

2.2.1.b.-Disfunció mitocondrial

Tòxics mitocondrials també condueixen a un patró d'atròfia similar a l'observat en el Huntington (Per revisió veure: Alexi i col., 1998a ; Sanberg i col., 1999). L'àcid 3-nitropropiònic (3-NP) i el malonat (MA) són toxines mitocondrials que interfereixen en la síntesi d'ATP inhibint la succinat deshidrogenasa que és activa en el cicle de l'àcid tricarboxílic, en el complex II de la cadena de transport d'electrons. La lesió amb 3-NP (o MA) proporciona un model animal per la malaltia de Huntington que reprodueix els aspectes de la malaltia en humans, en termes de patologia i simptomatologia (per a revisió veure: Borlongan i col., 1997). L'administració sistèmica del 3-NP mostra una pèrdua de neurones de projecció GABAèrgica amb una preservació relativa de les

neurones NADPHd acompanyada per una astrogliosis (Beal i col., 1993a; Brouillet i col., 1995; Nishino i col., 1996; Guyot i col., 1997).

2.2.1.c.-Models de ratolins transgènics de Huntington

Més recentment s'han establert uns ratolins transgènics que contenen una expansió de la regió amb repeticions CAG (Mangiarini i col., 1996; Davies i col., 1997; Martindale i col., 1998). Ratolins que normalment no tenen variacions en el número de repeticions CAG en el gen *huntingtin*, reben una porció de gen expandit de huntingtin humà. En ratolins mutants amb més de 100 repeticions, el gen i la proteïna s'expressen amb patrons normals (Mangiarini i col., 1996; Davies i col., 1997). A les 9-11 setmanes d'edat aquests ratolins mostren moviments coreics, tremolor, estereotípies involuntàries i convulsions induïdes per estímuls. Els ratolins es moren a les 10-13 setmanes d'edat. Tot i que els cervells són més petits que de normal, no s'evidencia una pèrdua neuronal del nucli estriat ni d'altres regions. També s'han realitzat estudis en ratolins transgènics injectats amb l'àcid kaínic per estudiar el paper de l'excitotoxicitat en la malaltia de Huntington (Morton i col., 2000).

3.-REACCIÓ GLIAL

Les úniques funcions del sistema nerviós central s'han atribuït a les propietats de les seves cèl·lules excitables, les neurones. Existeix, però, una altra classe de cèl·lules en el sistema nerviós, que es coneix com glia o neuroglia. Clàssicament, les cèl·lules glials han sigut considerades com cèl·lules no excitables. La glia del sistema nerviós central està formada principalment per astròcits, oligodendròcits, i microglia, mentre que en el sistema nerviós perifèric està format per les cèl·lules de Schwann i les cèl·lules satèl·lit ganglionars. Actualment està ben establert que les cèl·lules glials representen unes companyes de les neurones a llarg de la seva vida. Per exemple, durant la neurogènesis i

el desenvolupament, les cèl·lules glials aporten recolzament per la migració de les neurones i dels cons de creixement, un procés mediat per la síntesi i la secreció d'una gran varietat de factors de creixement i components de la matriu extracel·lular. En l'adult, les cèl·lules glials mantenen l'homeostasis neuronal, la plasticitat sinàptica i la reparació.

No totes les cèl·lules glials responen de la mateixa forma o al mateix temps després de que hagi tingut lloc una lesió neuronal. El cas més notable és el dels oligodendròcits, formadors de mielina, els quals per un cantó no mostren canvis reactius després de la lesió del sistema nerviós central i a més se semblen molt a les neurones podent-se observar la seva susceptibilitat després d'un dany sostingut (Ludwin, 1997). Això implica que els astròcits i la microglia són les dues poblacions principals de cèl·lules glials reactives.

3.1.-ASTRÒCITS

Els astròcits són cèl·lules estrellades amb múltiples processos fins, alguns dels quals estan associats a les parets capil·lars (Peters i col., 1991). Aquestes cèl·lules glials ocupen un gran volum en el sistema nerviós central. Dades estructurals i funcionals suggereixen que, degut a les seves característiques citològiques, els astròcits juguen un paper central en la distribució de substractes energètics de la circulació a les neurones (Golgi, 1886; Tscopoulos, 1996). El seu paper és efectuar diverses funcions, entre les quals s'inclouen el suport cel·lular durant el desenvolupament del sistema nerviós central, l'homeostasi de ions, la recaptació d'alguns neurotransmissors, la recaptació de CO_2, així com la contribució al sistema immunitari del cervell i la neuromodulació (Parpura i col., 2000; Anderson i col., 2000). També preserven la integritat del teixit després d'una agressió.

Pel bon funcionament de la transmissió sinàptica, és imprescindible dur a terme un control de l'entorn de les neurones. Alguns astròcits es disposen al voltant de l'espai sinàptic i recapten l'excés de glutamat després de la comunicació sinàptica. Els astròcits capten el glutamat mitjançant un sistema intercambiador depenent de Na^+ i K^+ (per revisió: Anderson i Swanson, 2000). El glutamat entra en l'astròcit acompanyat de Na^+ i

surt un K^+. En el funcionament normal del sistema, els astròcits regularien la concentració de K^+ extracel·lular que es produeix durant la neurotransmissió, expulsant-lo cap a l'endoteli vascular, quan es produeix un desequilibri del sistema i la concentració de K^+ extracel·lular és molt elevada, la captació de glutamat pot deixar de funcionar, acumulant-se glutamat, que en excés causarà excitotoxicitat, tant en neurones com en astròcits, ja que aquests també presenten receptors pel glutamat.

En cultius cel·lulars s'ha descrit la importància dels astròcits en el metabolisme del glutatió, si aquest mecanisme no funciona es produeix una disminució de l'efecte de defensa dels astròcits per les neurones front els RLO (radicals lliures d'oxigen). De fet recentment s'ha descrit una possible relació del sistema de glutatió en desordres neurològics (per revisió: Dringen, 2000)

L'astrogliosis representa una resposta universal a tots els tipus de lesió del sistema nerviós central, tot i que les característiques associades a aquesta reacció cel·lular varia d'un o altre tipus de lesió (Norton i col., 1992). En la primera resposta, la funció dels astròcits estaria encaminada a mantenir els diferents equilibris alterats. Posteriorment, els astròcits es tornen reactius, s'hipertròfien i poden proliferar.

Existeixen diferents marcadors que ens indiquen si existeix una reacció astrogial. Els més coneguts són: la proteïna glial fibrilar àcida (GFAP), la Vimentina i l'S-100b. Aquestes tres proteïnes s'utilitzen per identificar astròcits, tant en cervell intacte com en lesions del sistema nerviós central (Eng i col., 1995; Boyes i col., 1986). La GFAP és un filament intermedi que s'expressa constitutivament en astròcits associat a fibres de la substància blanca. La reactivitat astroglial implica canvis en la citoarquitectura dels astròcits; es produeix una hiperplàsia cel·lular, i els filaments intermedis veuen incrementada la seva expressió. Després d'una lesió en el cervell la quantitat de GFAP, així com el número de cèl·lules GFAP positives incrementa (Jabs i col., 1999). Lesions realitzades amb l'MPTP en ratolins van trobar un increment ràpid de la GFAP, sense trencament de la barrera hematoencefàlica (O'Callaghan i col., 1990). D'aquesta forma, aquest increment de la GFAP és un dels indicadores més sensible per a la lesió del cervell.

Una altra característica que es pot observar després d'una lesió en el cervell, seria la disposició dels astròcits en forma de cicatriu al voltant de la lesió. El paper d'aquesta

barrera no es coneix, s'ha suggerit la possibilitat de que fos un intent del sistema nerviós central de restaurar l'homeostasis aïllant la regió danyada (Fith i Silver, 1997), tot i que també s'ha intentat explicar la falta de regeneració del sistema nerviós central per la formació d'aquesta cicatriu glial i la seva associació amb la matriu extracel·lular, que estarien interferint en la reparació neuronal o en la regeneració axonal (Steeves i Tetzlaff, 1998). Existeixen molts treballs encaminats a l'estudi de l'etiologia de l'astrogliosis i els mecanismes mitjançant els quals es podria reduir la barrera glial (per revisió: McGraw i col., 2001).

Els astròcits, de la mateixa manera que les neurones, expressen proteïnes de l'estrés de la família HSP (Heat Shock protein). La HSP27 s'expressa majoritàriament en astròcits, tot i que també es pot trobar en algunes poblacions neuronals, bàsicament motoneurones (Plumier i col., 1997a; b). La HSP27 podria associar-se a l'actina dels astròcits, preservant d'aquesta forma el citoesquelet. S'ha descrit una inducció d'aquesta proteïna a nivell dels astròcits després de lesions induïdes per una isquèmia (Kato i col., 1994) o l'àcid kaínic (Plumier i col., 1996).

3.2.-LA MIGROGLIA

La cèl·lula microglial, descrita originalment per Del Rio-Hortega (1932), forma part del sistema fagocític mononuclear i reconeguda com a macròfags residents en el cervell (Perry i Gordon, 1989). Es creu que la microglia deriva de les cèl·lules precursores de la medul·la òsea (Ling i Wong, 1993; Perry i Gordon, 1991).

La morfologia de les cèl·lules de la microglia varia en funció del seu estat funcional (Streit i col, 1988). En el desenvolupament postnatal la microglia adquireix una forma ameboide. Són cèl·lules amb gran capacitat de moviment i proliferació. Progressivament es produeix una maduració i la microglia ameboide és substituïda per la microglia ramificada (Imamoto i Leblond 1978). Aquesta microglia ramificada es troba tan en la substància gris com en la substància blanca del cervell de rata adulta.

3.2.1.-Interacció microglial-neuronal en la lesió

La microglia en repòs es transforma ràpidament en un estat activat en resposta a un ampli ventall d'estímuls. L'activació microglial té lloc via un patró estereotípic de molts passos que comprèn una seqüència comuna de resposta cel·lular (fig.6). **Primer pas**: les cèl·lules microglials proliferen i migren al punt de lesió, on pateixen canvis morfològics, immunofenotípics i funcionals, sense arribar a tenir característiques fagocítiques (Streit i col., 1989b; Gehrmann i col., 1995; Gehrmann y Kreutzberg, 1995). Les alteracions immunfenotípiques inclouen un increment de diversos receptors de membrana i citoquines, que pot ajudar a descriure l'estat actiu de la microglia. Entre aquests canvis s'inclouen l'increment de l'expressió del receptor del complex de tipus 3 (CR3) i la inducció de l'antígen del complex major d'histocompatibilitat de classe I (MHCI). Els canvis funcionals de microglia activada inclouen l'alliberació d'una gran varietat de substàncies mediadores que inclouen proteases citotòxiques, RLO, intermediaris nitrogenats i excitotoxines (Colton i Gilbert, 1987; Banati i col., 1993a; Gehrmann i col., 1995). **Segon pas:** la seva transformació en macròfags fagocítics del cervell, procés que té lloc si existeix una degeneració neuronal extensa (Streit i Kreutzberg, 1988). **Tercer pas:** Procés d'eliminació encara desconegut, presumptament a través de un mecanisme programat (i.e. apoptosis) (Gerhmann i col., 1995, Gehrmann i Banati, 1995). Aquesta hiposensibilitat de la resposta immune, que segueix a la lesió, a través de l'eliminació de les cèl·lules glials és un mecanisme de control molt important a fi de limitar la imflamació local i prevenir l'extensió del dany al teixit sa.

Existeixen diferents marcadors per identificar els diferents estats de la microglia. Mitjançant l'anticòs OX-42 es pot marcar el receptor del complement de tipus 3 (CR3), que s'expressa en la microglia en estat de repòs, i que, com ja s'ha mencionat incrementa la seva expressió en la microglia activa i en la fagocítica. Per diferenciar la microglia reactiva i la fagocítica front la microglia ramificada en repòs s'utilitza l'OX-6, marcador del complex major d'histocompatibilitat de classe I (MHC I) (Kiefer i Kreutzberg, 1991).

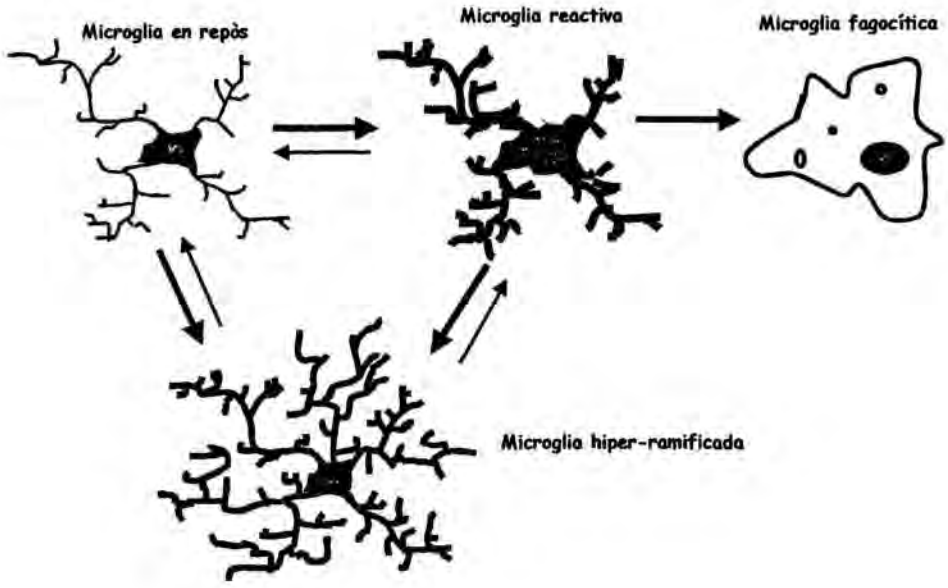

Fig.6.-Plasticitat funcional de la microglia.

Molts treballs han estudiat el reclutament ràpid de les cèl·lules microglials al punt de lesió ja sigui excitotòxica (McGeer i col., 1993; Akiyama i col., 1994), isquèmica (Lees, 1993; Kato i col., 1994) o traumàtica (Dusart i Schwab, 1994) i s'han identificat vàries senyals candidates a participar en l'activació microglial. Les citocines com la interleucina-1β, l'interferó-γ i el factor tumoral necròtic (TNF), sembla ser que són factors importants, degut a que afecten a la proliferació i a canvis immunfenotípics/funcionals en cultius de cèl·lules microglials (Suzumura i col., 1990; Loughlin i col., 1992; Gehrmann i col., 1995)

Generalment s'ha pensat que la microglia del voltant de la lesió està involucrada en eliminar els residus de les neurones que degeneren (Rozemuler i col., 1989, Moller i col., 1996), però la seva exacta contribució encara no és ben coneguda. Per un costat s'ha hipotetitzat que la microglia incrementa la lesió neuronal, mitjançant la síntesi i secreció de diferents agents que potencien una sobreactivitat sinàptica, i incrementa la lesió primària (McGeer i col., 1988; Itagaki i col., 1989; Giulien i col., 1995). Per altra

banda, existeix la possibilitat de que l'activació microglial sigui un mecanisme neuroprotector, amb el fi de limitar la neurodegeneració i millorar la recuperació sinàptica després de la lesió (Batchelor i col., 1999). A més, la microglia pot protegir els sistemes neuronals alliberant factors neuroprotectors com el factor bàsic de creixement fibroblàstic (bFGF) i el factor de creixement nerviós (NGF) (Araujo i Cotman, 1992). A part del seu paper en la modificació del dany neuronal, la microglia activa pot contribuir a recuperar i reconstruir la sinapsis (Lazarov-Spiegler i col., 1996; Moller i col., 1996; Prewity col., 1997).

3.2.3.-Receptor Benzodiacepínic Perifèric.

Les cèl·lules glials es caracteritzen per expressar, a nivell de la membrana mitocondrial, el receptor benzodiacepínic perifèric (PBR) que s'ha proposat com un marcador del dany neuronal (Benavides i col., 1987; Guilarte i col., 1995). Increments en la seva densitat s'han apreciat utilitzant tècniques de binding i autoradiogràfiques a nivell de les zones lesionades després de diferents insults tals com l'isquèmia global (Stephenson i col., 1995) o l'àcid domoic (Kulmann i Guitarte, 1997). Donada la important proliferació astroglial, així com l'activació microglial associada als processos de dany neuronal i degeneració és lògic pensar que els increments en els PBRs poden ser atribuïbles a la gliosis i per això que pugui constituir un bon indicador del dany neuronal. Estudis autoradiogràfics realitzats per Stephenson i col., (1995) van permetre determinar que el PBR colocalitzaba amb la microglia reactiva després d'un episodi isquèmic, mentre que els astròcits, situats en la zona que envolta la lesió, no presentaven marcatge. Amb tot això podem concloure que el PBR constitueix un marcador del dany neuronal i que la seva quantificació, mitjançant lligams específics, pot considerar-se un mètode per valorar els processos danyins del sistema nerviós central.

4.-ELS FACTORS TRÒFICS

4.1.-INTRODUCCIÓ

Els factors tròfics són proteïnes endògenes solubles que regulen la supervivència, el creixement, la plasticitat o la síntesi de proteïnes per funcions diferenciades de les neurones. D'aquesta definició s'exclouen molècules lligades a la membrana que afecten a l'adhesió i supervivència neuronal així com molècules no proteiques com hormones esteroïdees i gangliòsids (Hefti i col., 1993).

Els factors neurotròfics promouen la supervivència neuronal durant el desenvolupament. S'ha proposat que els axons competeixen per quantitats limitants de factors neurotròfics produïts pels teixits diana (Grimes i col., 1996; Yuen i col., 1996). Les neurones que no aconsegueixen obtenir una quantitat suficient de factor neurotròfic necessari moren mitjançant un procés anomenat mort cel·lular programada (Thoenen i col., 1987; Connor i Dragunow, 1998). Es creu que aquest procés regula el número de neurones i les connexions durant el desenvolupament del sistema nerviós central. A més, en l'adult, els factors neurotròfics són necessaris per mantenir les funcions i els fenotípics neuronals específics (Blesch i col., 1998). A més, també representen un medi potencial per modificar la disfunció neuronal, activació astrocítica i reaccions inflamatòries en condicions patològiques. Un gran número de dades suggereixen que alguns factors neurotròfics, sota certes condicions, també modulen la plasticitat neuronal que sorgeix durant l'edat i sota condicions traumàtiques degeneratives (Blesch i col., 1998).

Resultats recents suggereixen que les alteracions dels nivells neurotròfics poden estar implicades en la fisiopatologia de les malalties neurodegeneratives, com són les malalties de Parkinson i Alzheimer (Connor i Dragunow, 1998).

4.2.-MECANISME D'ACCIÓ DELS FACTORS TRÒFICS

El control de la supervivència i del creixement neuronal necessita la síntesi i l'alliberació dels factors tròfics, així com la distribució de receptors específics per aquests factors. El mecanisme fisiològic d'alliberació per aquestes proteïnes amb propietats neurotròfiques, encara no ha sigut establert. Sembla possible que algunes d'aquests proteïnes sols s'alliberen després de produir-se una lesió. Una cop alliberades, és necessari que els factors s'uneixin a receptors específics que es localitzen en les neurones que responen per produir els seus efectes. Aquests receptors són diferents depenent del factor tròfic de què es tracti.

Una cop lligat al receptor, el factor tròfic juntament amb el receptor serien internalizats i transportats fins al cos neuronal on exercirien la seva funció, **transport retrògrad**. A part del seu efecte sobre el cos neuronal s'ha descrit un efecte local de les neurotrofines a nivell dels espais pre- i postsinàptics, en fenòmens de plasticitat sinàptica (Berninger i Poo, 1996). El fet de que algunes poblacions neuronals expressin tan la neurotrofina com el seu receptor d'alta afinitat, ha dut a suggerir un possible **efecte autocrí** de les neurotrofines en aquestes poblacions. A més, cèl·lules veïnes, ja siguin glials o neuronals, poden ser capaces d'aportar suport tròfic mitjançant un mecanisme conegut com **efecte paracrí**. Finalment s'ha demostrat que paral·lelament al seu efecte retrògrad les neurotrofines poden **transportar-se anterògradament** en la neurona productora cap al seu axó. En aquest model la neurona capaç de respondre a les neurotrofines seria innervada per la neurona productora que secretaria la neurotrofina en les seves terminacions sinàptiques, i serien les regions postsinàptiques les que captarien, via el seu receptor, les neurotrofines.

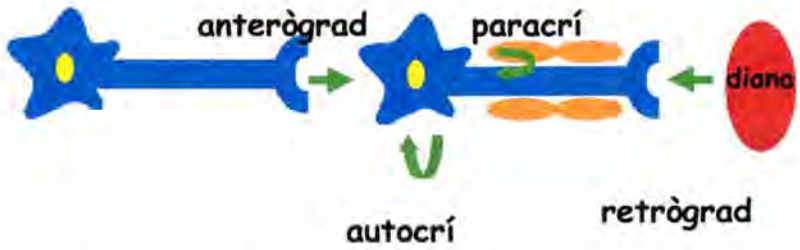

Fig.7.-Mecanisme d'acció dels factors tròfics

4.3.- LES NEUROTROFINES I ELS SEUS RECEPTORS.

4.3.1.-Les neurotrofines

La família de les neurotrofines està formada pel factor de creixement nerviós (NGF) (Levi-Montalcini i Hamburger, 1953), el factor nerviós derivat del cervell (BDNF) (Barde i col., 1982), la neurotrofina-3 (NT-3) (Maisonpierre i col., 1990), la neurotrofina-4/5 (NT-4/5) (Ip i col., 1992), la neurotrofina-6 (NT-6) (Götz i col., 1994) i la neurotrofina-7 (NT-7) (Lai i col., 1998; Nilsson i col., 1998). Encara no es coneix molt bé el paper de l'NT-6 i de l'NT-7.

L'NGF va ser el primer factor neurotròfic identificat (Levi-Montalcini i Hamburger, 1953). Ha sigut reconegut com el prototip de substàncies que promouen el manteniment i la supervivència mitjançant mecanismes de transport retrògrad (Hendry i col., 1974). L'NGF va ser parcialment purificat a partir de la glàndula submaxilar del ratolí mascle (Cohen, 1960; Levi-Montalcini, 1987a, b).

El BDNF fou el segon membre de la família de l'NGF aïllat (Bade i Edgar, 1982). Presenta un 50% d'homologia en les seves seqüències amb l'NGF (Maisonpierre i col., 1990). El BDNF actua sobre poblacions neuronals que no responen a l'NGF (Barde i Edgar, 1982). L'homologia estructural entre el BDNF i l'NGF suggerí l'existència d'altres molècules relacionades. Amb estudis de PCR (reacció en cadena de la polimerasa) i altres tècniques sofisticades de clonatge, es van identificar altres molècules relacionades amb l'NGF. L'NT-3 fou la següent (Enfors i col., 1990, Hohn i

col., 1990; Maisonpierre i col., 1990a), conté 199 aminoàcids i presenta un 57% d'aminoàcids idèntics amb l'NGF i un 58% amb el BDNF. Tot i que presenta aquesta homologia d'estructures, la seva activitat biològica és diferent i les seves característiques espai-temporals també .

4.3.2.-Els receptors per a les neurotrofines.

Les accions biològiques de les neurotrofines són mediades per dues classes de receptors (Per revisió veure: Barbacid, 1994a, 1994b; Chao, 1994; Dechant i col., 1994; Bothwell, 1995). Totes les neurotrofines s'uneixen amb similar afinitat ($Kd \approx 10^{-9}M$) a una proteïna, coneguda com el receptor de les neurotrofines p75 ($p75^{NTR}$). A més, les neurotrofines s'uneixen amb diferent selectivitat a tres receptors tirosina quinasa altament relacionats, coneguts com els receptors d'alta afinitat ($Kd \approx 10^{-11}M$) de les neurotrofines, el trk A, el trk B i el trk C. L'NGF s'uneix específicament al trk A, el BDNF i l'NT-4/5 poden activar el trk B (Klein i col., 1992; Klein i col., 1994), mentre que l'NT-3 preferentment activa el trk C (Lambelle i col., 1991), però també té l'habilitat d'activar el trk A i el trk B (Huang, 1999).

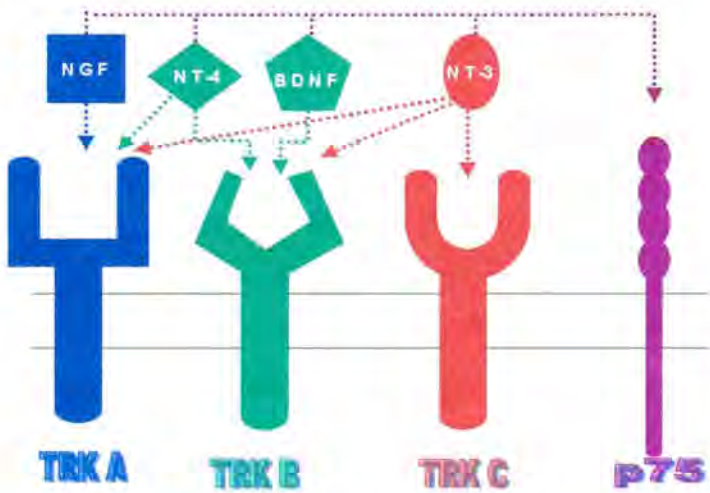

Fig.8.- Els receptors de les neurotrofines, els receptors Trk

4.3.2.a.-El receptor p75

El receptor p75 és una glicoproteïna transmembrana i és un membre de la superfamília TNFR/fas/CD40 coneguts com a dominis de mort (Baker i Reddy, 1998). Conté una porció extracel·lular amb quatre grups rics en cisteïna involucrada en la unió amb el lligant, una regió transmembrana, i una seqüència citoplasmàtica curta sense activitat catalítica (Chao i col., 1994; Bothwell, 1995; Ibañez, 1998).

Els mecanismes de transducció que medien els efectes biològics del $p75^{NTR}$ en neurones encara no són ben coneguts. Per un cantó, pot modular les respostes cel·lulars de les neurotrofines interaccionant amb el receptor d'alta afinitat. La modulació de la interacció del receptor trk amb les neurotrofines ha sigut considerada com el principal mecanisme d'acció del $p75^{NTR}$ des del descobriment dels receptors trk (Barbacid, 1994a, 1994b; Chao i col., 1994; Bothwell, 1995). També s'ha descrit la seva participació en el transport axonal retrògrad de les neurotrofines des de les regions diana (von Bartheld i col., 1996). En canvi, es va demostrar clarament que el $p75^{NTR}$ podia induir respostes cel·lulars en absència dels receptors trk, quan era activat per l'NGF (Carter i Lewin, 1997; Frade i Barde, 1999). En alguna situació pot induir la mort cel·lular notable.

4.3.2.b.-El receptor Trk

La caracterització de un oncogén aïllat d'un carcinoma de colon (Martin-Zanca i col., 1986) va dur a la identificació del proto-oncogén corresponent, que codifica per una glicoproteïna de membrana de 140 kDa amb un domini citoplasmàtic proteïna-tirosina quinasa (Martin-Zanca i col., 1989).

Degut a la seva estructura, l'oncogen es va designar *trk*, que consistia en dues seqüències fusionades que codificaven per una isoforma de tropomiosina i un receptor tirosina quinasa. Es va establir que la proteïna per la que codificaba era el receptor d'alta afinitat que mediava les principals accions de l'NGF, el trkA (Kaplan i col., 1991). Posteriorment s'han identificat altres gens molt relacionats i que s'expressen abundanment en el cervell, són el *trkB* (Klein i col., 1989) i el *trkC* (Lamballe i col.,

1991) que codifiquen per les proteïnes que uneixen al BDNF, l'NT-4/5 i l'NT-3 (Klein i col., 1991, Lamballe i col., 1991, Squinto i col., 1991)

Anàlisis moleculars i bioquímics han revelat la presència de diferents variants dels receptors trk. D'aquests, els més predominants semblen ser les formes truncades del trkB i del trkC, que s'expressen en concentracions considerables en el cervell adult (Barbacid, 1995; Middlemas i col., 1991; Tsoulfas i col., 1993). El receptor trk de cadena complerta té una estructura complexa que inclou una regió transmembrana, una porció extracel·lular compromesa amb la unió amb la neurotrofina i una porció intracel·lular amb activitat tirosina quinasa. La regió extracel·lular inclou un domini ric en leucina flanquejat per dos grups de cisteïnes, seguits de dos dominis tipus immunoglobulines (Schneider i Schwieger, 1991), una de les quals és essencial per la unió de la neurotrofina (Urfer i col., 1995; Haniu i col., 1997; Ultsch i col., 1999).

La unió neurotrofina-receptor porta l'activació del receptor trk de cadena complerta mitjançant un procés de dos passos, que consisteix en la inducció del lligam de la dimerització del receptor i la autofosforilació dels residus de tirosina de la regió intracel·lular (Schlessinger i Ullrich, 1992). El receptor actiu està preparat per interaccionar i fosforilar vàries dianes intracel·lulars (Segal i Greenberg, 1996). Els receptors truncats són idèntics al receptor funcional en el seu domini extracel·lular i transmembrana però no presenten la seqüència de la tirosina quinasa citoplasmàtica. Aquests receptors poden unir les neurotrofines però no poden activar els mecanismes de senyals intracel·lulars. S'han buscat funcions alternatives per aquests receptors, moltes de les quals són les mateixes que es postulen com a funcions del $p75^{NTR}$:

a) Presentació del lligam. b) Internalització. c) Activació de mecanismes intracel·lulars alternatius. d) Modulació de la resposta de les neurotrofines (segrestant els receptors amb propietats catalítiques). e) Mediació de les interaccions cèl·lula-cèl·lula.

L'NGF exerceix el seu efecte sobre poblacions neuronals molt restringides, coincidint amb la localització dels llocs de unió d'alta afinitat per l'NGF així com amb la localització de l'ARNm pel *trk*A. Contràriament al que succeeix amb el *trk*A, els transcrits pel *trk*B i pel *trk*C es troben distribuïts àmpliament en tot el cervell (Merlio i col., 1992). Estudis de hibridació *in situ* mostren que les formes complertes del *trk*B i del *trk*C s'expressen (i molts vegades es co-expressen) en la majoria de les neurones del

sistema nerviós central, però no en cèl·lules no-neuronals com astròcits, oligodendròcits, cèl·lules ependimàries i cèl·lules dels plexes coroidals. Les formes truncades del *trk*B i del *trk*C es troben molt expressades en el sistema nerviós central, amb la forma truncada del *trk*B localitzada també en cèl·lules no-neuronals. El fet de que el *trk*B i el *trk*C es trobin tan expressats en el sistema nerviós central, així com l'expressió també molt àmplia dels seus lligants (Maisonpierre i col., 1990b), suggereix que les funcions del BDNF i de l'NT-3 en el sistema nerviós central són molt més diverses que les funcions de l'NGF (Lindsay i col., 199). L'expressió també molt elevada de les formes truncades pel *trk*B i pel *trk*C (no *trk*A) suggereix que han d'existir mecanismes per restringir la distribució o per modular l'acció del BDNF i de l'NT-3 després de ser alliberades dels seus llocs de síntesi.

4.3.3.-Accions biològiques de les neurotrofines en els circuits substància negra-nucli estriat-escorça cerebral

4.3.3.a.-El BDNF

El BDNF ha sigut el factor més estudiat al llarg d'aquests darrers anys, i ha estat considerat com un factor molt important en la supervivència i diferenciació neuronal. S'han realitzat diferents treballs sobre la seva distribució en el cervell humà, les modificacions de la seva expressió i la distribució en desordres neurodegeneratius. Els estudis més rellevants en humans han estat recollits en la revisió de Murer i els seus col·laboradors (2001).

Sobre la via nigroestriada s'ha demostrat que el BDNF prevé la mort espontània de neurones dopaminèrgiques en cultius primàris mesencefàlics de rata (Hyman i col., 1991; Knusel i col., 1991), i que protegeix les neurones TH immunoreactives de la toxina selectiva MPP^+ (Hyman i col., 1991). Aquests resultats van fer créixer l'interés per el possible us com agent terapèutic per la malaltia de Parkinson del BDNF. Aquests estudis van estimular la recerca per determinar si l'administració del BDNF podia protegir les neurones dopaminèrgiques de un dany induït per toxines o l'axotomia *in*

vivo. Així doncs existeixen treballs on descriuen l'efecte neuroprotector del BDNF sobre el dany produït per la injecció de la 6-OHDA (Schultz i col., 1995), l' MPTP (Hung i Lee, 1996) o per l'MPP^+ (Frim i col., 1994; Galpern i col., 1996).

Cal destacar treballs on es mostra que el BDNF promou la diferenciació fenotípica i la supervivència de les neurones estriatals GABAèrgiques contra l'excitotoxicitat *in vitro* (Mizuno i col., 1994; Ventimiglia i col., 1995) i *in vivo* (Martínez-Serrano i Björklund, 1996; Pérez-Navarro i col., 1999).

Efectes tròfics autocrins i paracrins també s'han descrit en cultius corticals embrionaris de rata (Gosch i col., 1994), si afegien anticossos contra el BDNF al medi de cultiu disminuïa la supervivència de les neurones corticals. A més el BDNF també regulava el fenotip i la funció de neurones GABAèrgiques en cultius corticals de rata (Widmer i Hefti, 1994; Pappas i Parnavelas, 1997). A part d'aquests treballs sobre la supervivència del cultiu s'han realitzat estudis per determinar si el BDNF exògen protegia el cultiu cortical de lesions la majoria de ells mostraren un efecte beneficiós del BDNF (Shimonhama i col., 1993; Chen i Mattson, 1994; Chen i col., 1994; Takei col., 1999; Tremblay i col., 1999). També l'administració d'aquesta neurotrofina protegeix aquestes neurones de la ischemia (per revisió Nikolics, 1999) i de la lesió excitotòxica (per revisió Lindholm, 1994).

4.3.3.b.- L' NT-3

Al contrari del BDNF i l'NGF els nivells de l'ARNm per l'NT-3 en el sistema nerviós central és més alt durant el desenvolupament fetal i disminueix en el cervell adult. Aquests nivells més elevats en el cervell fetal comparat amb altres neurotrofines pot indicar que l'NT-3 té un paper més important en la supervivència i diferenciació neuronal durant el desenvolupament (Enfors i col., 1990; Maisonpierre i col., 1990).

La primera demostració que el gen de l'NT-3 codificava per l'activitat neurotròfica va ser descrit per Maisonpierre i col. (1991). Particularment s'ha observat que l'NT-3 és essencial per la supevivència de les neurones simpàtiques i sensorials que més tard seran dependents de l'NGF o del BDNF (Fariñas i col., 1994).

Sobre les neurones dopaminergiques del mesencèfal s'ha observat que l'NT-3 incrementa la supervivència (Gall i col., 1992), incrementa l'activitat TH i el numero de neurones GAD positives en cultiu mesencefàlics embrionàries de rata (Hyman i col., 1994).

A més s'ha observat que l'NT-3 incrementa el número de neurones calbindina positives així com les neurones GABAèrgiques en cultius estriatals de rata (Ventimiglia i col., 1995) i protegeix les neurones estriatals de la hipoglucèmia (Nakao i col., 1995). *In vivo* s'ha observat que quan s'administren cèl·lules que secreten aquesta neurotrofina, aquesta regula el fenotip de les neurones estriatals de projecció (Perez-Navarro i col., 1999).

Sobre l'escorça cerebral l'NT-3 té un efecte dual en les arboritzacions dendrítiques (McAllister i col., 1997) i afecta també de forma diferent l'arborització axonal en diferents capes corticals (Castellani i Bolz, 1999).

En el cervell de rata s'ha observat que en alguns paradigmes hi ha un increment del BDNF i de l'NGF mentre que hi ha un disminució dels nivells de ARNm de l'NT-3 en els mateixos (Lindvall i col., 1994; Rocamora i col., 1993; Takeda i col., 1992). Això implica que l'NT-.3 està involucrada en diferents accions biologiques que el BDNF i l'NGF i queda per saber quin és el seu paper després d'una lesió en el cervell.

4.3.3.c.- L'NT-4/5

L'ARNm de l'NT-4 s'expressa amb uns nivells molt més baixos que les altres neurotrofines. Timmusk i col., (1993) van trobar l'expressió de l'ARNm en tots els teixits i regions del cervell examinat excepte del moll de l'os utilitzant la tècnica de la riboprotecció. Apart dels canvis observats durant el desenvolupament l'expressió d'aquesta neurotrofina sembla estar menys influenciada per els canvis de l'entorn que afecten l'expressió de les altres neurotrofines ja que molts estímuls que regulen l'expressió de les altres neurotrofines en el cervell no afecten l'NT-4, potser degut als seus nivells baixos d'expressió (Ibañez, 1996).

Les accions biològiques de l'NT-4/5 s'han estudiat *in vitro* utilitzant cultius primaris de diferents subpoblacions neuronals i també s'han realitzat estudis *in vivo* on

s'injecta l'NT-4/5 o s'implanten cèl·lules que expressen alts nivells d'aquesta neurotrofina.

S'ha observat que en cultius de neurones dopaminèrgiques incrementa l'activitat de l'enzim TH i el seu contingut de dopamina (Hyman i col., 1994), a més en promou el creixement dendrític (DeFazio i col., 2000) i influencia la seva diferenciació morfològica (Studer i col., 1995). L'NT-4/5 també prevé la mort de les neurones dopaminèrgiques produïda per l'MPP^+ (Hynes i col., 1994) i l'òxid nítric (Lingor i col., 2000). Estudis *in vivo* han mostrat que l'NT-4/5 protegeix les neurones dopaminèrgiques de la substància negra després de l'axotomia de la via nigroestriada (Hagg, 1998).

Sobre les neurones estriatals promou la supervivència i la diferenciació, i presenta accions tròfiques sobre les neurones GABAèrgiques de l'estriat (Widmer i Hefti, 1994; Ardelt i col., 1994; Ventimiglia i col., 1995). També incrementa la supervivència de les neurones colinèrgiques estriatals (Abiru i col., 1996). La seva administració en el cervell incrementa els nivells de l'ARNm de la taquiquinina en les neurones striatonigrals i reverteix la pèrdua de neurones parvalbumina positives deguda a l'acció de l'àcid quinolínic (Alexi i col., 1997).

Quan s'administra crònicament en cultius corticals incrementa la recaptació de GABA i les neurones marcades per GABA son més grans (Widmer i Hefti, 1994). L'NT-4/5 també modula l'expressió de l'NPY en l'escorça cerebral (Widmer i col., 1998) en cultius organotípics. I protegeix del dany produït per la deprivació de glucosa sobre aquestes neurones (Cheng i col., 1994). S'ha mostrat també que actua sobre el creixement dendrític de diferents capes corticals (McAllisteri col., 1997).

4.3.4.-Knockouts per a les neurotrofines i els seus receptors.

L'obtenció d'animals transgènics, als quals els manca algun gen per a alguna neurotrofina, han aportat noves dades en quant a la importancia d'aquestes substàncies per el desenvolupament i supervivència d'alguns tipus neuronals. En els animals que els manca el gen per BDNF provoca dèficits importants en la coordinació. La supervivència de les neurones simpàtiques, de les neurones DAèrgiques del mesencèfal i de les motoneurones no es veu afectada, en canvi si que es veu greument afectada la

supervivència de diferents tipus de neurones sensorials. A més, es formen totes les capes de l'escorça cerebral, tot i que son més primes que en els control. Els sistema nerviós central no presenta canvis estructurals importants, però si que s'han observat alguns canvis en l'expressió del NPY i d'algunes proteïnes que uneixen calci, com la calbindina, el que suggereix que la funció d'aquestes neurones es veu afectada (Jones i col., 1994; Ernfors i col., 1994).

L'absència de l'NT-3 provoca greus dèficits en neurones sensitives i simpàtiques mentre que el sistema nerviós central i també les motoneurones sembla que es desenvolupen amb normalitat (Fariñas i col., 1994).

En els animals que els falta el gen per l'NT-4/5 s'han observat també dèficits en algunes poblacions de neurones sensorials mentre que les motoneurones no es veuen afectades i tampoc s'observa pèrdua de neurones dopaminèrgiques de la substància negra (Liu i col., 1995).

Tots aquests resultats, al igual que els obtinguts en animals que els manca el gen per algun dels receptors de les neurotrofines, mostren que existeix una interacció entre aquests factors a l'hora de regular la supervivència i funció d'algunes poblacions neuronals. També s'han obtingut alguns animals deficitàris alhora dels gens per el BDNF i per l'NT-4/5. Aquests animals presenten una pèrdua més important en algunes poblacions de neurones sensorials comparat amb els animals als quals els manca només una d'aquestes neurotrofines (Conover i col., 1995; Liu i col., 1995). En els animals que els manca el gen el *trk*B hi ha una reducció important en el nombre de les motoneurones (Klein i col., 1993) mentre que en els animals deficitaris del BDNF i l'NT-4/5 no s'observen canvis (Conover i col., 1995; Liu i col., 1995). Aquests resultats suggereixen que alguna altra neurotrofina que actua sobre el receptor *trk*B, com per exemple l'NT-3 pot estar implicada en el manteniment d'aquestes neurones.

Com ja s'ha comentat, també s'han desenvolupat animals transgènics als que els manca algun dels gens que codifiquen per aquests receptors i s'ha pogut observar que aquests receptors són essencials per a la supervivència d'algunes neurones que responen a les neurotrofines a través d'aquests receptors i no per altres, suggerint que moltes de les neurones es troben regulades per la interacció de vàries neurotrofines. En els animals que no tenen el receptor *trk*A s'ha observat una greu afectació de les neurones del

sistema nerviós perifèric però en canvi no s'han observat canvis en el número de neurones del sistema nerviós central que responen a l'NGF com són les neurones colinèrgiques de projecció i les neurones estriatals (Smeyne i col., 1994). La delecció del gen que codifica pel receptor *trk*B provoca la mort dels animals pocs dies després del seu naixement i presenten un nombre reduït de neurones motores i sensorials (Klein i col., 1993). Finalment, els animals que no tenen el gen pel *trk*C presenten una resposta motora anormal. Aquests animals presenten un dèficit neuronal del 20% en el gangli de l'arrel dorsal i també la pèrdua dels aferents Ia que innerven els feixos musculars (Klein i col., 1994). Sorprenentment, tot i que els ARNm pel *trk*B i pel *trk*C es troben àmpliament distribuïts en el sistema nerviós central, no s'han observat defectes importants en els animals que no tenen el *trk*B (Klein i col., 1993) o el *trk*C (Klein i col., 1994). L'expressió d'aquests dos gens és coincident en algunes regions el que suggereix que poden estar involucrats en vies funcionalment redundants.

OBJECTIUS

L'objectiu general d'aquest treball és aprofundir en l'estudi de la fisiopatologia de les malalties degeneratives, un dels objectius principals per a la farmacologia actual degut a l'increment de la seva incidència en les últimes dècades.

Mitjançant la utilització de models experimentals d'aquestes patologies s'han plantejat diferents objectius més concrets:

A.- Induir la malatia de Parkinson experimental a través de la injeció de l'MPP^+ en la substància negra de rata.

1.-Estudiar la resposta glial en el nucli estriat després de la degeneració anteròg rada de les neurones dopaminèrgiques causada per la injecció de l'MPP+ en la substància negra. Implicació en el mecanisme de mort neuronal i de regeneració.

B.- Induir la malaltia de Huntington experimental a través de la injecció d'aminoàcids excitadors en el nucli estriat de rata.

1.-Caracteritzar la resposta endògena tròfica a l'excitotoxicitat, valorant els canvis en l'expressió del BDNF i l'NT-3 així com la dels seus receptors, TrkB i TrkC, en l'escorça cerebral despres de la injecció de diferents agonistes del receptor del glutamat en el nucli estriat.

2.-Estudiar la regulació endògena dels nivells de BDNF en la substància negra de rata després de la lesió estriatal induïda per l'àcid kaínic, així com la possible implicació d'aquesta neurotrofina en la supervivència de les neurones de la substància negra front la lesió excitotòxica en el nucli estriat.

3.-Estudiar el possible efecte neuroprotector de les neurotrofines BDNF, NT-3 i NT-4/5, sobre les diferents poblacions neuronals de projecció del nucli estriat, en el model excitotòxic de l'àcid quinolínic. Es van implantar en l'estriat de rata adulta línees cel·lulars establertes que secreten alts nivells de BDNF, NT-3 i NT-4/5 recombinant abans de la injecció de l'aminoàcid excitador.

RESULTATS

MPP^+ INJECTION INTO RAT SUBSTANTIA NIGRA CAUSES SECONDARY GLIAL ACTIVATION BUT NOT CELL DEATH IN THE IPSILATERAL STRIATUM

Publicat a la revista Neurobiology of Disease 7, 343-361 (2000)

Neurobiology of Disease 7, 343–361 (2000)
doi:10.1006/nbdi.2000.0308, *available online at http://www.idealibrary.com on* IDEAL®

MPP$^+$ Injection into Rat Substantia Nigra Causes Secondary Glial Activation but Not Cell Death in the Ipsilateral Striatum

Anna M. Canudas,* Bibiana Friguls,*,† Anna M. Planas,†
Cecília Gabriel,* Elena Escubedo,* Jordi Camarasa,*
Antoni Camins,* and Mercè Pallàs*,[1]

**Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari de Pedralbes, E-08028 Barcelona; and †Departament de Farmacologia i Toxicologia, IIBB-CSIC, IDIBAPS, Jordi Girona 18-26, 08034 Barcelona, Spain*

Received May 15, 2000; accepted for publication May 15, 2000

Injection of MPP$^+$ into the substantia nigra causes extensive necrosis and anterograde degeneration of pars compacta dopaminergic neurons. We studied secondary effects in the ipsilateral striatum by examining dopaminergic terminals, signs of neuronal damage, and glial reactivity at 1, 2, 3, and 7 days after injection of MPP$^+$ into the substantia nigra. Dopaminergic terminals and uptake sites were evaluated with [^{3}H]GBR-12935 binding and tyrosine hydroxylase immunoreactivity. Glial reaction was examined with markers of astrocytes and microglia. Stereology was used to evaluate any changes in neuronal density. Tyrosine hydroxylase immunoreactivity and [^{3}H]GBR-12935 binding markedly decreased (74%) from days 2 to 7. Loss of dopaminergic terminals in the ipsilateral striatum was accompanied by an intense astroglial and, to a lesser extent, microglial reaction. However, no signs of cell damage, neuronal loss, or disruption of the blood–brain barrier were found in the striatum. Resident astroglial and microglial cells showed a morphological shift and notable changes in protein expression typical of glial reactivity, yet the presence of macrophage-like cells was not detected. This study shows that injection of MPP$^+$ in the substantia nigra causes a secondary reaction within the ipsilateral striatum involving the transformation of quiescent glia to reactive glia. It is suggested that stimuli derived from damaged dopaminergic terminals within the striatum are able to activate resident glia and that this glial transformation may promote repair and regeneration.

Key Words: **reactive glia; [^{3}H]PK 11195; binding; immunohistochemistry; rat.**

INTRODUCTION

Parkinson's disease is a common neurodegenerative disorder characterized mainly by tremor, muscular rigidity, slowness of movement, rigidity, and postural instability. This syndrome involves progressive degeneration of substantia nigra pars compacta neurons resulting in destruction of dopaminergic nigrostriatal pathways. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a remarkably selective neurotoxin, causes parkinsonism in humans (Langston *et al.*, 1983) and when administered to monkeys and mice it reproduces many of the biochemical and neuropathological changes in the nigrostriatal dopaminergic pathway found in human Parkinson's disease (Langston & Irwin, 1986; Kopin & Markey, 1988; Heikkila *et al.*, 1989).

MPTP crosses the blood–brain barrier after systemic injection. Once in the brain, MPTP is bioactivated by monoamino oxidase B, which is localized in astrocytes, to its active metabolite, 1-methyl-4-phenylpyridinium (MPP$^+$) (Chiba *et al.*, 1984; Markey *et al.*, 1984). Dopaminergic neurons accumulate MPP$^+$, which enters the cells via the dopamine uptake system. MPP$^+$ is concentrated inside the mitochondria, where it blocks

[1] To whom correspondence and reprint requests should be addressed. Fax: 34-934 035 982. E-mail: pallas@far.ub.es.

0969-9961/00 $35.00

complex I of the electron transport chain, which in turn decreases the production of ATP and increases the formation of free radicals such as superoxide (Mizuno *et al.*, 1987). It has been proposed that mitochondrial dysfunction, oxidative stress, and energy failure caused by MPP^+ may impair cell function, leading to cell death (Jakson-Lewis *et al.*, 1995; Tatton & Kish, 1997). MPTP administration or stereotaxic MPP^+ injection into the substantia nigra causes necrosis and decreases striatal dopamine and tyrosine hydroxylase protein in the nigrostiatal pathway (Sun *et al.*, 1988; O'Callahan *et al.*, 1990a, b; Neafsey *et al.*, 1995; Santiago *et al.*, 1995, 1996).

One of the responses of the CNS to brain damage is reactive gliosis. MPTP administration in mice causes large increases in an astrocyte-localized protein called glial fibrillary acidic protein (GFAP) in the nigrostriatal pathway associated with loss of tyrosine hydroxylase (O'Callaghan *et al.*, 1988, 1990a, 1992). Hypertrophy of astrocytes (Lindsay, 1986; Norton *et al.*, 1992) is characterized by lengthening and thickening of astrocyte processes and is most often accompanied by proliferation of microglia. Resident microglia show a quiescent, extremely ramified morphology under physiological conditions in the adult brain. Resting microglial cells upon pathological stimulation show a dramatic change in morphology that is associated with acquisition of reactivity, thus functionality (Streit *et al.*, 1988; Perry and Gordon, 1988). However, the stimuli and the process by which resting microglia become reactive remains unclear. Reactive microglia are involved in numerous neuropathological conditions in man, as they mediate immune responses (Graeber and Streit, 1990), and become activated after brain damage (Lehrmann *et al.*, 1997). Furthermore, several studies have shown microglial activation after loss of nerve cell ends due to neuronal degeneration in a distant brain region (Gehrmann *et al.*, 1991; Fagan and Gage, 1994), thus suggesting that degenerating terminals induce microglial reactivity.

Here we examined whether anterograde degeneration of dopaminergic neurons caused by injection of MPP^+ in the substantia nigra resulted in glial reactivity in the ipsilateral striatum, and whether the effect was associated with neuronal loss in this area. Histological and stereological analyses together with immunohistochemical and pharmacological studies were carried out in order to detect signs of neuronal loss, and to find evidence of glial reaction.

MATERIALS AND METHODS

Animals and Treatments

Male Sprague–Dawley rats, weighing 210–250 g, were used for all the experiments. Care and use of these animals followed the policy on the use of animals in neuroscience research, published by the Society for Neuroscience. The protocols were approved by a review committee of the University of Barcelona under the supervision of the "Generalitat de Catalunya." Animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic instrument (David Kopf, Palo Alto, CA). MPP^+ iodide (RBI Inc., Natick, MA) was dissolved in saline solution at concentrations of 28, 56, and 84 $\mu g/\mu l$ (pH 7.4). 1 μl of these solutions was injected into the left substantia nigra (coordinates A: −0.28, L: −0.25; DV: −0.8 from dura; Pellegrino *et al.*, 1967). The right striatum was used as a control.

Binding studies. Animals (n = 3–4 per time point) were killed by decapitation at 2 or 7 days after MPP^+ treatment. The brain was immediately removed from the skull and both striata were quickly excised. Tissues were weighed and homogenised in 10 volumes of cold buffer (0.32 M sucrose and 5 mM Tris–HCl, pH 7.4) with a Polytron (Kinematica). For synaptosomal preparation the homogenate was filtered through a nylon mesh screen and centrifuged at 1,000g for 10 min. The supernatant was centrifuged twice at 35,000g for 15 min. The final pellet was dissolved in assay buffer (50 mM Tris–HCl, 120 mM NaCl, pH 7.7) with a Potter–Elvejheim homogenizer. For crude mitochondrial membrane preparations homogenates were centrifuged twice at 15,000g for 30 min. Finally the pellet was dissolved in 50 mM Tris–HCl buffer, pH 7.4, with a Potter–Elvejheim homogeniser. Protein content was determined (Bradford, 1976) using bovine serum albumin (BSA) as the standard.

Equilibrium binding assays for [^{3}H]GBR-12935 were performed at room temperature for 45 min. The final volume in each tube was 1 ml, including 30 μg of protein/tube, [^{3}H]GBR-12935 (sp act: 40 Ci/mmol, New England Nuclear) in a concentration range of 0.1–3 nM and BSA (0.01%). Specific binding was defined as the difference between the radioactivities measured in the absence (total binding) and in the presence (nonspecific binding) of bupropion (30 μM). Samples were filtered under vacuum over Whatman GF/B glass fiber filters soaked in 0.5% polyethyleneimine. Filters were rapidly washed three times in 4 ml of ice-cold Tris–HCl buffer and placed in vials con-

taining 10 ml of Biogreen 1 scintillation cocktail (Scharlau S.A., Spain). Radioactivity was measured by liquid scintillation spectroscopy in a Beckman LS-1800 (Beckman Instruments Inc. Fullerton, CA) with an efficiency of 45%. Affinity constant (K_D) and maximal binding site (Bmax) were calculated using RADLIG 4.0 program (Biosoft, Cambridge, UK). The significance was calculated using ANOVA and subsequent Tukey's test.

Peripheral benzodiazepine binding site density was measured by equilibrium binding assays at 0–4°C using [^{3}H]PK11195 (sp act: 85 Ci/mmol, New England Nuclear). Assays were performed in a final volume of 0.25 ml (pH 7.4) including 125 μl of [^{3}H]PK11195 in a concentration range of 0.5–15 nM and 125 μl of protein preparation (100 μg of protein per assay). Specific binding was defined as the difference between the radioactivities measured in the absence (total binding) and in the presence (non-specific binding) of 10 μM of unlabeled Ro 5-4864. After incubation for 120 min reaction was stopped, the radioactivity was measured (efficiency: 40%), and calculations were performed as described for [^{3}H]GBR-12935 experiments.

Immunohistochemistry. Animals were anaesthetised with ether and perfused through the heart with heparinized saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (100 ml/100 g body weight). Brains were removed and postfixed overnight in the same solution. Some of the brains (n = 9) were embedded in paraffin and sectioned (5-μm coronal) with a microtome, while the remaining (n = 20) were directly sectioned (50-μm coronal) with a vibratome, and kept at −20°C in a cryoprotective solution for further procedures. Immunohistochemistry was performed free-floating with vibratome sections as reported (Soriano *et al.*, 1994), using the following antibodies at the concentration indicated in parentheses: OX-42 (1:500) a monoclonal antibody against complement receptor-3 (Serotec), which recognizes resting and reactive microglia/macrophages (Robinson *et al.*, 1986); OX-6 (1:1000) (Serotec) a mouse monoclonal antibody wich recognises MHC class II antigens and is used as a marker of reactive microglia/macrophages (Gehrmann *et al.*, 1991, 1992); a monoclonal antibody against GFAP (1:500) (Boehringer), a sheep polyclonal antibody against albumin (1:1000) (Serotec) to detect changes in blood–brain barrier permeability (Reynolds and Morton, 1998), and antibodies against two stress proteins: the 27-kDa heat shock protein HSP27 (1:4000) (Stressgen) (Kato *et al.*, 1994; Plumier *et al.*, 1996), and the 72-kDa heat shock protein HSP72 (1:200) (Oncogene) (Planas *et al.*, 1997). In brief, endogenous peroxidases were blocked with 3% hydrogen peroxide and 10% methanol in PBS. Sections were incubated for 2 h with either 3% normal serum or 5% BSA to reduce nonspecific binding. They were then washed in T-PBS (phosphate buffered saline (PBS) containing 0.5% Triton-X100) and incubated overnight at 4°C with the primary antibody. Thereafter, the sections were rinsed in T-PBS, incubated for 1 h with a biotinylated secondary antibody (1:200, Vectastain, Vector), followed by 1% avidin–biotin–peridoxidase complex (ABC kit, Vector Laboratories). Finally, the sections were washed in T-PBS and PBS, and the immunoreaction was developed with 0.05% diaminobenzidine and 0.03% H_2O_2 in PBS.

Double immunohistochemistry was carried out by incubating the sections with a second primary antibody as described (Planas *et al.*, 1998). The first immunoreaction was designed to label astroglia with GFAP, and the second reaction was to label microglia with the monoclonal antibody against OX42 as above. After extensive washing following the first immunoreaction, sections were incubated with 3% of normal horse serum for 2 h to block unspecific binding sites for OX-42 immunohistochemistry. Sections were incubated overnight at 4°C with the corresponding primary antibody, followed by treatment with biotinylated secondary antibody (1:200), and the ABC complex. Finally, sections were washed in 0.01 M sodium phosphate buffer, pH 6, preincubated in 0.01% benzidine dihydrochloride and 0.025% sodium nitroferricyanide in 0.01M sodium phosphate buffer for 10 min and developed in the same solution containing 0.005% of H_2O_2.

Additionally, paraffin sections were used for hematoxylin and eosin stain, for histochemistry with biotinylated tomato lectin (2.5 μg/ml) for microglial detection (Planas *et al.*, 1998) and for immunohistochemistry with the following mouse monoclonal antibodies: a mouse monoclonal antibody against tyrosine hydroxylase (1:200) (Chemicon International, Inc.) (Soriano *et al.*, 1997), and an antibody against proliferating cell nuclear antigen (PCNA, 1:400) (Oncogene) (Planas *et al.*, 1998).

Assessment of any secondary cell death in the ipsilateral striatum. Histological examination in the striatum was carried out for each rat, including those killed at 1, 2, 3, and 7 days after MPP^+ injection, in sections that were consecutive to those used for immunohistochemical studies. Paraffin sections were stained with hematoxylin and eosin, and vibratome sections were stained with cresyl violet. Sections were examined under the light microscope looking for any signs of

cellular and/or tissular damage in the ipsilateral striatum. Several sections from the substantia nigra of each treated rat were also stained with hematoxylin or cresyl violet in order to assess damage in this area.

In addition, the presence of cells bearing fragmented DNA was examined in the striatum with in situ labelling of DNA fragmentation with the tunel technique, as previously reported by the authors (Ferrer *et al.*, 1996). This study was carried out on the same paraffin sections as those used for immunohistochemistry mentioned above, which were obtained at 1, 3, and 7 days after MPP^+ injection in the ipsilateral substantia nigra. Positive controls for the tunel technique were paraffin sections from the gerbil hippocampus at 4 days after transient global cerebral ischemia, as we previously reported (Ferrer *et al.*, 1995).

Moreover, search for degenerating neurons was carried out at 1, 2, 3, and 7 days using a fluorochrome, Fluoro-Jade, which has been reported to stain degenerating neurons (Schmued *et al.*, 1997; Freyaldenhoven *et al.*, 1997; Bowyer *et al.*, 1998; Kakaia *et al.*, 1998). Staining was performed as essentially described by Schmued *et al.* (1997). In brief, vibratome sections were mounted in gelatine-coated slides, dried, immersed in 100% ethanol, 70% ethanol, distilled water, followed by 0.06% potassium permanganate (Sigma) for 15 min, and washed with distilled water and transferred to a Fluoro-Jade solution (0.1 mg/ml) (Histo-Chem Inc., Jefferson, AR) for 30 min at RT. Slides were then washed with distilled water, rapidly dried, immersed in xylene, and cover-slipped with DPX mounting media (Sigma). Labeling was observed under the fluorescent light microscope using an appropriate filter system (FITC). Rat brains obtained 1 day after the administration of kainic acid (9 mg/kg, i.p), and after a transient episode of middle cerebral artery occlusion (Planas *et al.*, 1997) were used as positive controls for Fluoro-Jade staining. Double labelling against neurons or glia and Fluoro-Jade was carried out as follows. First, immunohistochemistry with antibodies against neuron-specific nuclear marker or Hsp27 was carried out using the ABC method, as described above, sections were then mounted in gelatine-coated slides and histochemistry for Fluoro-Jade was carried out. Observation of the sections under the visible light showed a single immunohistochemistry stain for neuron-specific antibody or Hsp27. Fluorescence light revealed a bright Fluoro-Jade stain, while the immunoreaction was seen as a dark stain.

Finally, we also aimed to detect whether cell loss in the striatum occurred as a result of the degeneration of the ipsilateral substantia nigra. Neuronal cells were counted and their density was estimated in the ipsilateral striatum with the aid of stereological tools (Gundersen *et al.*, 1988a, b). This study was performed in two additional groups of rats ($n = 6$ per group) at 7 days after injection of 84 μg MPP^+ in the left substantia nigra, as detailed above, and in controls. All the brains used in this experiment were first examined for the presence of glial reaction in the ipsilateral striatum by means of immunohistochemistry. Rats were transcardially perfused with 4% paraformaldehyde as before, and serial 50-μm coronal sections were cut with a vibratome. Twelve series of sections were taken though the striatum and the distance between each other was 300 μm. Our aim was to count neurons, rather than total cells, because of the glial reaction. Accordingly, sections were subjected to immunohistochemistry with an antibody that selectively stains neuronal nuclei (mouse monoclonal antibody against neuron-specific nuclear protein, Chemicon, diluted 1:1000). The immunohistochemical procedure was as detailed above for mouse monoclonal antibodies. Sections were examined under a light microscope (×100) that was connected to an image analysis system through a video camera and stereology was carried out using an appropriate software (AIS, Imaging Research Inc., Canada). Four areas were randomly taken within the ipsilateral striatum for neuronal counting in each section (Fig. 12). Then 12 sections per rat (taken every 300 μm), by 6 rats per group, by 2 groups, control and MPP^+, were used for neuronal counting (Fig. 12). Striatal neuronal density was estimated by a sampling strategy called the dissector, which has been suggested a direct estimator of the number of 3-D objects, unbiased by their shape and size (Sterio, 1984; Gundersen *et al.*, 1988b). This method involves the application of a sampling frame with an area of 26,000 square pixel (12,000 μm^2) and a grid matrix of 52 by 52 pixels. Immunohistochemical images showing neuronal nuclei were segmented without any other criteria than a density threshold (the segmentation range was set from 0.2747 to 0.3668 optical density units). The sampling frame, which is a tool with two inclusive and two exclusive boundaries (Fig. 12), was then randomly applied using a celluloid grid that split the captured area in 9 equivalent regions. Cell counting was carried out in one of these nine regions randomly selected with the aid of a random number table. One target was accepted only when it was completely inside the frame or when touched by the inclusive boundary but not the exclusive boundary. In theory, the sampling frame provides all

targets with the same chance of being sampled. Neuronal density (N_v) was then estimated as the number of 3-D targets (N) per volume sampled (V_s) (The AIS Operations Manual, Imaging Research Inc., Ontario, Canada), and results are given as the number of neuronal nuclei per mm^3 in the ipsilateral striatum. As we analyzed the sections of one animal, we checked that the accumulated mean N_v value was stable. Thus the number of sections that we decided to evaluate sufficed to ensure reproducibility in the individual measurements. The coefficient of error of N was estimated for each individual as the ratio of the square root of the total variance to the mean. The total variance was calculated from the sum of the variance due to sampling within sections, i.e., the Nugget Variance, and that due to sampling between sections, i.e., arising from systematic random sampling of the section series (Avendaño and Lagares, 1996; West *et al.*, 1996). In addition, the striatal volume was estimated for each rat by integrating areas of the twelve coronal sections using morphometry software (AIS). A researcher (BF) that was not aware of the treatment given to rats carried out the stereological analysis. Results were statistically analyzed with one-way analysis of variance.

RESULTS

Behavior

Animals treated with MPP^+ showed postural abnormality and lack of mobility. Straub tail reaction and contralateral turning behavior were observed after the treatment. Decrease in body weight (15%) attributable to akinesia was detected after 7 days.

Degeneration of the Substantia Nigra

Administration of MPP^+ to the substantia nigra caused severe cell loss and degeneration within the area of injection (Fig. 1). The lesion was apparent 24 h postinjection and affected not only the substantia nigra pars compacta but also the neighbouring zone including pars reticulata (Figs. 1C and 1E). Within 24 h, a loss of tyrosine hydroxylase immunoreactivity was apparent in the ipsilateral substantia nigra (Fig. 2). The stress protein, Hsp70, was induced around the lesion from 1 to 7 days after MPP^+ injection (not shown).

Alterations in the Ipsilateral Striatum

Loss of dopaminergic terminals on corpus striatum was evident at all MPP^+ doses and times of death, as revealed by [^{3}H]GBR-12935 binding. Decreases (74%) in Bmax for [^{3}H]GBR-12935 were statistically significant at 2 and 7 days after administration of 28, 56, and 84 μg of MPP^+ ($P < 0.001$, Table 1). K_D for [^{3}H]GBR-12935 after MPP^+ was not significantly different from controls. Moreover, the B_{max} in the cortex of MPP^+ treated rats was not different from that of controls (1634 $\pm$ 17.6 and 1455.6 $\pm$ 28.7 fmol/mg protein, respectively), showing the lack of effect of MPP^+ on this brain region. This effect was accompanied by loss of tyrosine hydroxylase immunoreactivity, which was detected in the ipsilateral striatum at 3 and 7 days after MPP^+ injection (Fig. 2).

Microglial Reaction in the Ipsilateral Striatum

Saturation analysis for [^{3}H]PK 11195 binding following MPP^+ administration showed increases in Bmax in the ipsilateral striatum compared with the contralateral (Fig. 3, Table 2). The increase was significant ($P < 0.001$) at 84 μg of MPP^+. K_D values after MPP^+ injections were not different from controls.

The quiescent ramified microglial cells that are normally present in the brain express the CR3 complement receptor and can therefore be labeled with an antibody (MRC OX42) against this protein. Injection of 84 μg of MPP^+ in substantia nigra increased OX42 labeling in the ipsilateral striatum after 2 and 7 days and in a lesser extent after 24 h (Fig. 4). Microglial cells showed a more intense stain in the ipsilateral striatum than in the contralateral hemisphere or the striatum of control rats. Normal quiescent microglia are highly ramified (Fig. 4). After MPP^+ injection, microglial cells in the striatum became more spherical with thick spines at 7 days, indicating microglial reactivity (Fig. 4). This morphology is different from that of round macrophage-like cells that appear during neuronal death, such as those observed in the infarcted region after focal cerebral ischemia (Fig. 4) (see Planas *et al.*, 1998). At a lower MPP^+ dose, 28 μg, spiny reactive microglial cells were detected in the ipsilateral striatum at 7 but not 3 days after injection to the substantia nigra (not shown). The microglial reaction was weaker at 28 μg than at 84 μg of MPP^+.

A further change in antigen expression was detected in reactive microglia of the striatum ipsilateral to the

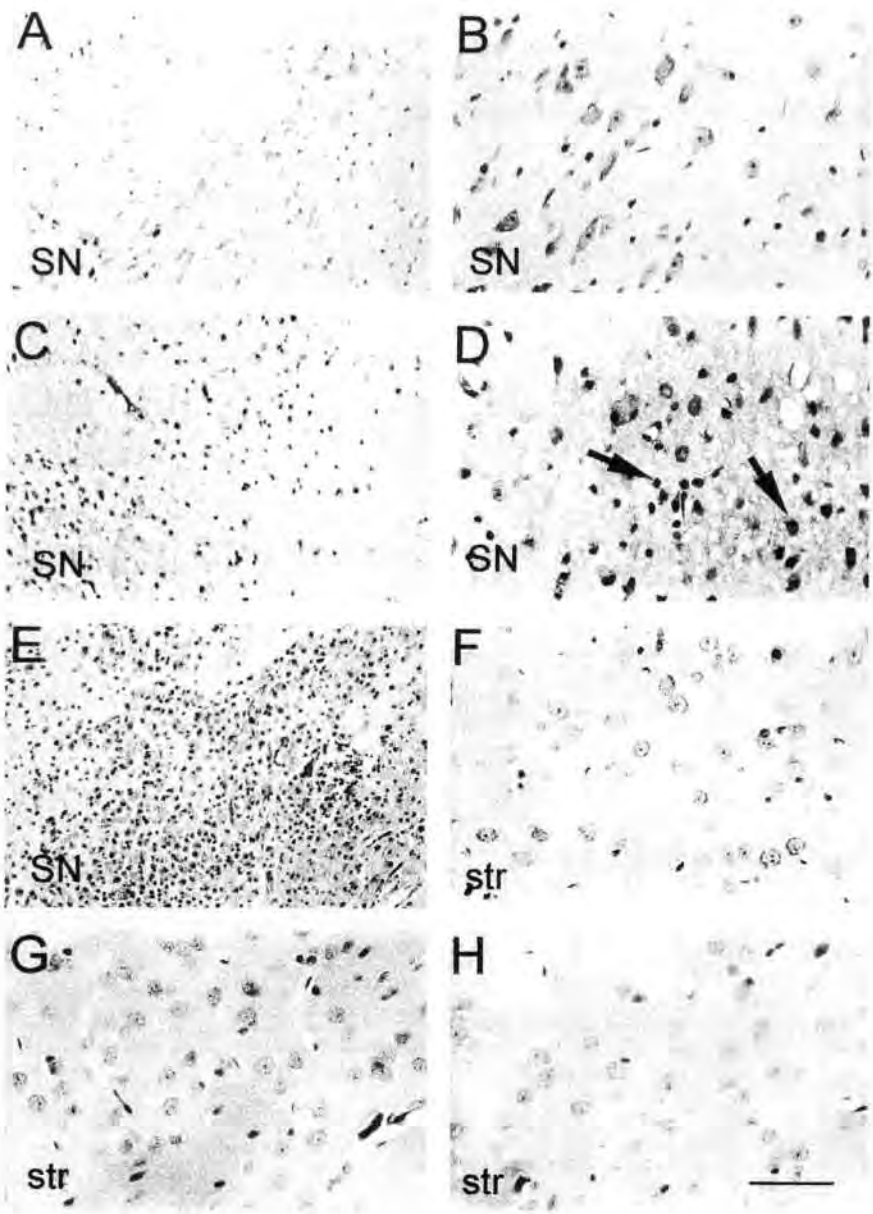

FIG. 1. Contralateral (A, B) and ipsilateral substantia nigra (C–E), and contralateral (F) and ispilateral (G, H) striatum at 3 (A–D, G) and 7 (E, F, H) days after MPP^+ injection, at a microscopic level. Extensive necrosis and glial reaction (arrows) is observed in the ipsilateral substantia nigra (C–E), whereas no morphological alterations were seen in the ipsilateral striatum (G, H). Hematoxylin and eosin. Bar: (A, C, E) 50 μm; (B, D, F–H) 25 μm. Neuronal-nuclei (arrow) in the ipsilateral MPP^+ and control. The MPP+ image shows the sampling frame. Bar: 100 μm. (D) Results of quantitative estimation of neuronal density showing no differences between groups. Values are expressed as mean ± SD.

substantia nigra lesion, coincidental with the change in microglia morphology. This change was due to induction of a specific antigen OX6, which is not normally expressed by quiescent microglia (Fig. 5). OX6 was detected in the ipsilateral striatum from 2 to 7 days after injections of 84 μg of MPP^+. At 28 μg of MPP^+, OX6 immunoreactivity was observed at 7 days, although the reaction was milder (Fig. 5D).

Astroglial Reaction in the Ipsilateral Striatum

Astrocytes became reactive in the ipsilateral striatum after the substantia nigra lesion as revealed by increased expression of GFAP (Fig. 6). GFAP stain revealed the network of astrocytic extensions surrounding blood vessels in the control (Figs. 6A and 6B) and ipsilateral striatum (Figs. 6C–6H). The high-

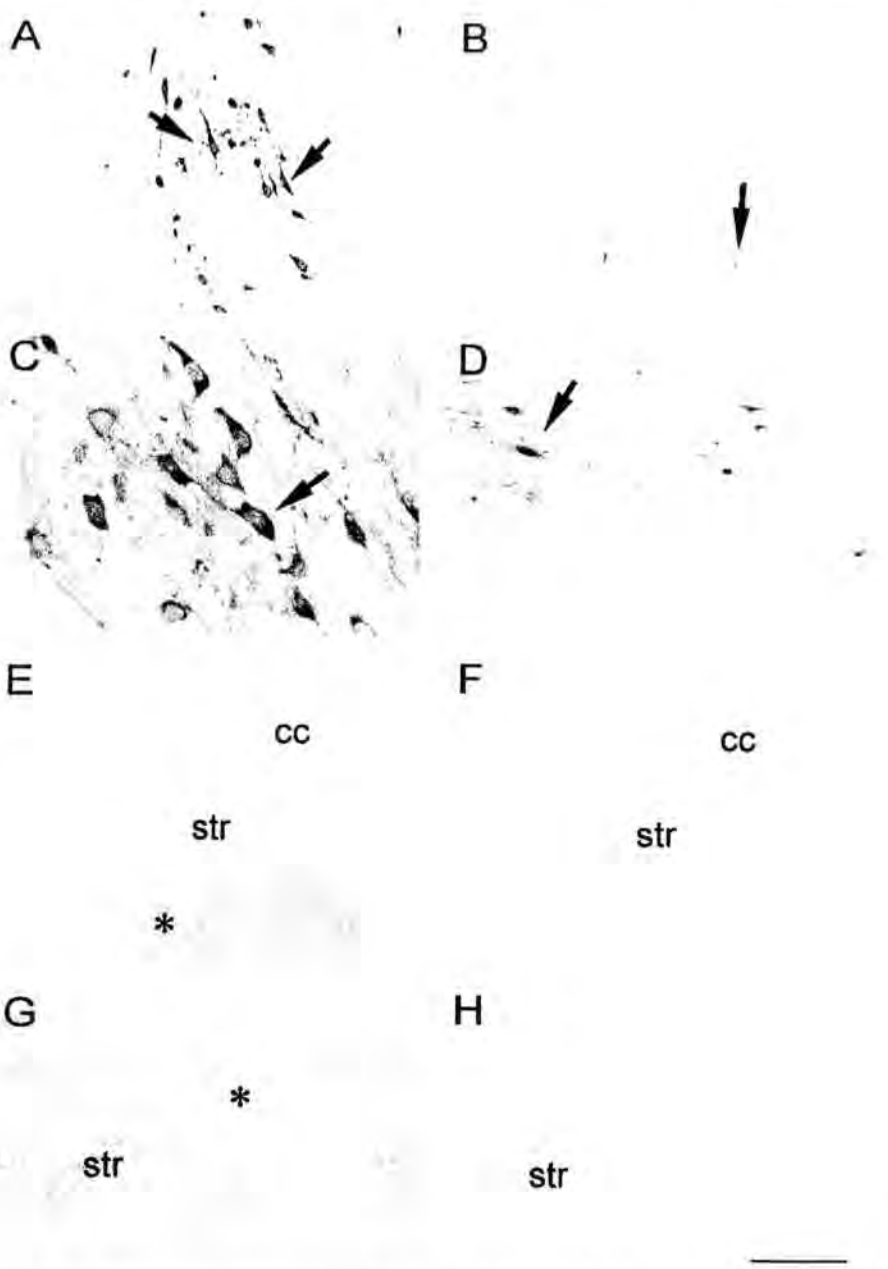

FIG. 2. Tyrosine hydroxylase immunoreactivity in contralateral (A, C) and ipsilateral (B, D) substantia nigra, and contralateral (E, G) and ipsilateral (F, H) striatum 7 days after MPP^+ administration in substantia nigra. Arrows point to immunoreactive dopaminergic neurons in substantia nigra pars compacta. Bar: (A, B, E, F) 50 μm; (C, D, G, H) 25 μm.

est intensity of stainnig was at 7 days after injection of 84 μg of MPP^+ (Figs. 6E and 6F). In addition, activated astrocytes expressed the stress protein Hsp27 (Figs. 7C–7H), which is not normally expressed in physiological conditions, but is highly inducible in astroglia after stressful conditions (Kato *et al.*, 1995; Plumier *et al.*, 1996). Here, substantia nigra lesion caused induction of Hsp27 in the ipsilateral striatum from day 1 after injection of MPP^+ and the reaction increased in the number of cells and intensity from days 2 (Figs. 7C–7D) to 7 (Figs. 7G and 7H). Low induction of astroglial Hsp27 was detected 7 days after the injection of 28 μg MPP^+ (not shown).

TABLE 1

K_d (nM) and B_{max} (fmol/mg protein) Values for [^{3}H]GBR 12935 Binding in Striatum of Control and Treated Rats by MPP$^+$

	48 h		1 week	
Treatment	K_D	B_{MAX}	K_D	B_{MAX}
Control	0.26 ± 0.10	6730 ± 555.10	0.44 ± 0.12	7655 ± 655.08
28 μg MPP$^+$	0.29 ± 0.09	2865.55 ± 38.50***	0.54 ± 0.22	2460.4 ± 169.15***
56 μg MPP$^+$	032 ± .004	2782.9 ± 32.19***	0.68 ± 0.22	2461.65 ± 64.80***
84 μg MPP$^+$	0.43 ± 0.07	2267.65 ± 21.85***	0.51 ± 0.10	1991.00 ± 263.65***

Note. Results are means ± SEM of 3–4 different rats run out in duplicate. ANOVA and Tukey's test: ***$P < 0.001$ vs control values.

Double immunohistochemistry against GFAP and OX42 showed that activated astrocytes colocalized with reactive microglia (Fig. 8).

Evaluation of Cell Proliferation

Proliferating cells express proliferating cell nuclear antigen (PCNA). Expression of PCNA in the adult rat brain was restricted to a few cells in the subventricular region (Fig. 9B). After the injection of 84 μg of MPP$^+$, faint PCNA staining was detected in a few scattered cells of the ipsilateral striatum at 3 and 7 days (Figs. 9C and 9D), together with the strong glial reaction.

Evaluation of Blood–Brain Barrier Integrity

Lack of blood–brain barrier integrity may be a sign of brain damage (Nordborg *et al.*, 1994). Albumin staining is used to detect whether the blood–brain barrier has been broken in pathological conditions, as the entry of albumin from the blood to brain is restricted in physiological conditions (Nordborg *et al.*, 1994; Reynolds and Morton, 1998). Here, we included in the study rats subjected to transient focal ischemia by middle cerebral artery occlusion for comparative purposes (Soriano *et al.*, 1997). Ischemic rats showed an increase in albumin staining in the infarcted cortex and striatum (Fig. 10D). Such effect was not detected in the ipsilateral striatum of rats whose substantia nigra had been injected MPP$^+$ (Figs. 10A–10C); no signs of albumin extravasation were thus apparent in the ipsilateral striatum after injection of MPP$^+$ in the ipsilateral substantia nigra.

Assessment of Signs of Neuronal Damage in the Striatum

No signs of striatal neuronal damage were detected under the light microscope in hematoxylin and eosin, or cresyl violet-stained sections (Fig. 1). Induction of the stress protein Hsp72 was neither detected in the striatum (not shown). Furthermore, DNA fragmentation staining was not observed in the ipsilateral striatum at 1, 3, or 7 days following MPP$^+$ injection (Figs. 9E and 9F), although the positive controls used in each experiment showed stained cells after the tunel reaction (Figs. 9G and 9H). Search for degenerating neurons in the ipsilateral striatum was completed by a fluorochrome, Fluoro-Jade (see Methods). Fluoro-Jade histochemistry was carried out at the striatum in vibratome sections obtained at 1, 2, 3, and 7 days after MPP$^+$ injection and in controls. Some background

TABLE 2

K_d (nM) and B_{max} (fmol/mg protein) Values for [^{3}H]PK 11195 Binding in Striatum of Control and Treated Rats by MPP$^+$

	48 h		1 week	
Treatment	K_D	B_{MAX}	K_D	B_{MAX}
Control	1.64 ± 0.23	405.13 ± 6.91	1.55 ± 0.24	394.38 ± 14.84
28 μg MPP$^+$	3.12 ± 0.62	528.12 ± 34.74	1.68 ± 0.30	533.62 ± 24.80
56 μg MPP$^+$	6.61 ± 1.48	537.88 ± 19.80	1.35 ± 0.11	555.12 ± 21.84^f
84 μg MPP$^+$	3.90 ± 0.40	722.4 ± 87.8**	2.92 ± 1.38	797.5 ± 143.18***^{f}

Note. Results are means ± SEM of 3–4 different rats run out in duplicate. ANOVA and Tukey's test: **$P < 0.01$, ***$P < 0.001$ vs control values; $^f P < 0.01$ vs 28 and 56 μg MPP$^+$ values.

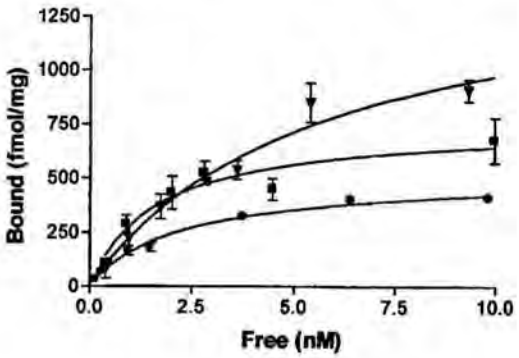

FIG. 3. Saturation analysis of [^{3}H] PK 11195 binding in control and MPP$^+$-treated rats killed at 48 h sacrifice post-treatment. (●) Control, (■) 28 μg MPP$^+$, (▼) 84 μg MPP$^+$. Results are the mean ± SEM from three or four rats.

stain was observed within striosomes of controls and MPP$^+$-treated rats (Figs. 11E–11H). No clear neuronal staining was detected in the striatum of MPP$^+$-treated rats (Figs. 11E and 11G), and no clear differences from controls were found (Figs. 11F and 11H). However, strong fluorescent labeling was detected in the positive controls: the hippocampus of rats injected with kainic acid (Figs. 11A and 11B) and the cortex of rats subjected to focal cerebral ischemia (Figs. 11C and 11D). The only appreciable difference between the ipsilateral and the contralateral striatum of rats injected MPP$^+$ was that in the former some fluorescence appeared in fibers within striosomes (Figs. 11E and 11G). Double labeling against the neuron-specific neuronal marker that was immunohistochemically stained with the ABC procedure (single staining as observed

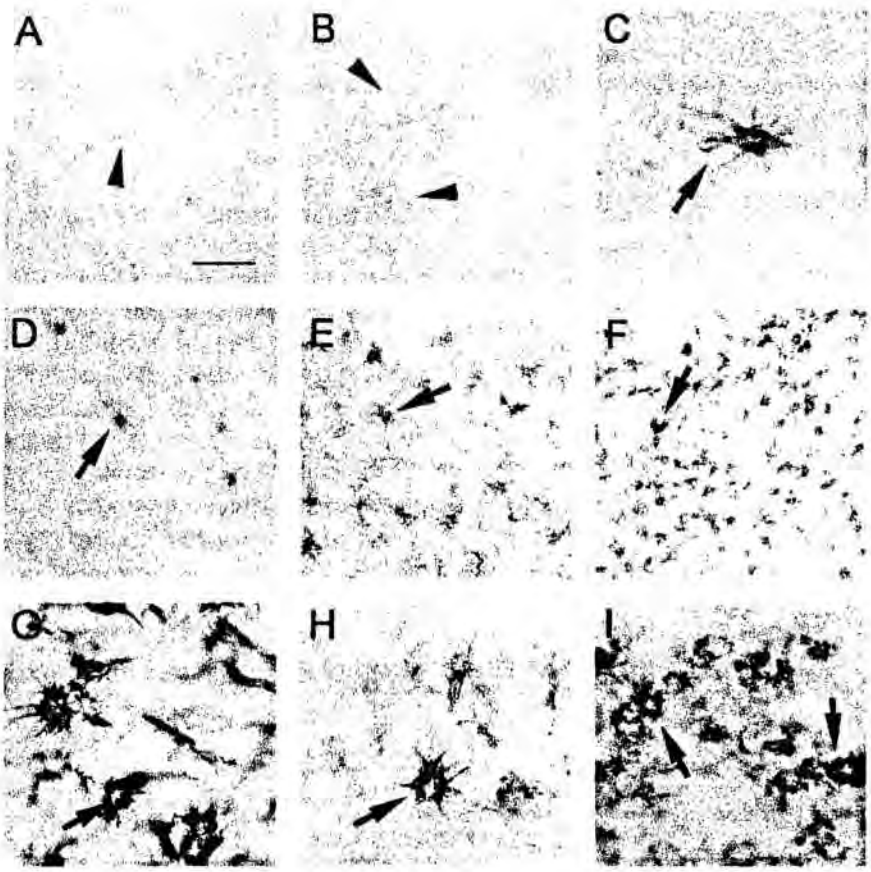

FIG. 4. Microglial cells in the striatum as revealed with OX42 immunoreactivity (arrows). Control (A, B) and 3 (E) and 7 (C, D, F–H) days after 28 μg (C, D) and 84 μg (E–H) MPP$^+$. Spiny reactive microglia strongly immunoreactive to OX42 are seen in the ipsilateral striatum after MPP$^+$ (G, H). A positive control obtained from striatum at 7 days after 1-h focal middle cerebral artery occlusion is included for the presence of round reactive microglia/macrophages (I). Bar: (A, D–F) 80 μm; (C, G–I) 25 μm.

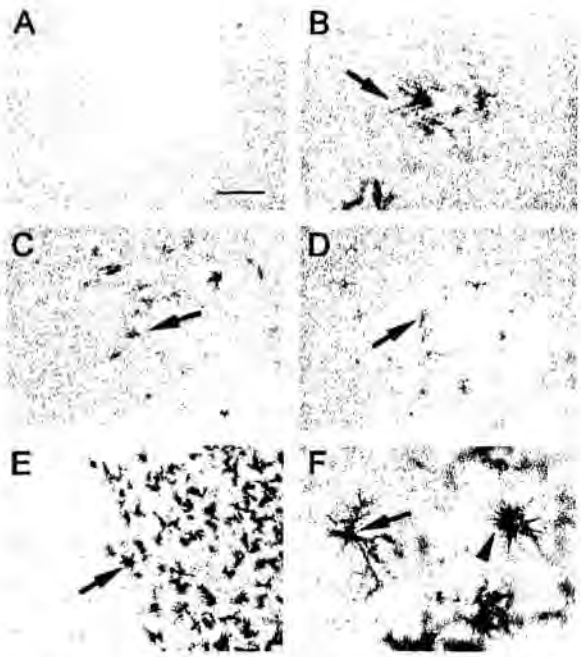

FIG. 5. Immunostaining against OX6 recognising MHC II antigens in the contralateral (A) and ipsilateral (B–F) striatum at 2 (B), 3 (C), and 7 (D–F) days after MPP^+ administration in the substantia nigra. At the dose of 84 μg (B, C, E, F) and 28 μg (D). Stained spiny microglial cells are detected in the ipsilateral striatum after MPP^+ (arrows). Bar: (A, C–E) 100 μm; (B, F) 25 μm.

with visible light is shown in Figs. 11J and 11L for ipsilateral and contralateral hemispheres) and Fluoro-Jade did not reveal any fluorescent staining associated with neurons (Figs. 11I and 11K for respective ipsilateral and contralateral hemispheres with fluorescent light). In addition, double labeling against Hsp27 (single staining as evidenced with visible light is shown in Fig. 11P for contralateral, and Figs. 11N, 11R, and 11T for the ipsilateral hemisphere at 2, 3, and 7 days after MPP^+) and Fluoro-Jade showed that reactive astrocytes surrounded the striosomes (Fig. 11O for contralateral, and Figs. 12M, 12Q, and 12S for ipsilateral MPP^+, as before).

Stereological Estimation of Neuronal Cell Numbers in the Ipsilateral Striatum

Stereological methods were followed in order to corroborate the previous results showing no signs of neuronal cell death in the ipsilateral striatum. Two groups of rats were studied: control and MPP^+-treated by injection in the substantia nigra 7 days earlier. All the MPP^+-injected rats showed glial reaction in the ipsilateral striatum, whereas the controls showed no reaction. Sections were immunostained with an antibody directed toward a neuron-specific nuclear protein (Fig. 12). Measurements were performed in each of the 12 coronal sections per rat, and 6 rats were studied per group. By this procedure we were able to detect differences in neuronal density between groups higher than 6%, which was the coefficient of variation within each group. Results are expressed as objects–neuronal nuclei-per mm^3 in the ipsialteral striatum. The mean ± SD estimated number of 3-D targets (N) within the sampled volume was 5115 ± 419 and 5173 ± 377 for controls and MPP^+-treated, respectively. The coefficient of error, which was lower than 0.05, assessed the precision of the estimates for each rat. The mean ± SD neuronal density was 33000 ± 1563 and 33017 ± 1933 neuronal nuclei per mm^3 for controls and MPP^+-treated, respectively (Fig. 12). The striatal volume was also estimated in order to assess any alterations due to edema or atrophy. The mean ± SD volumes were 23.2 ± 0.5 and 23.4 ± 1.7 mm^3 for controls and MPP^+-treated groups respectively. Statistical analyses showed no significant differences between groups for any of these parameters. Thus, the stereological method used here for counting neuronal nuclei did not evidence neuronal loss in the ipsilateral striatum after MPP^+ injection in the substantia nigra.

DISCUSSION

Injection of MPP^+ to the substantia nigra causes cytotoxic damage to nigrostriatal neurons, which is associated with loss of ipsilateral striatal dopamine (Sun *et al.*, 1988; Neafsey *et al.*, 1995). In the present study, injection of MPP^+ into substantia nigra caused not only selective dopaminergic toxicity in the pars compacta but was also cytotoxic to neighboring cells, which is consistent with previous reports (Ter Horst *et al.*, 1992). Subsequently, dopamine innervation in the ipsilateral striatum was reduced, as assessed with [^{3}H] GBR-12935, a selective dopamine uptake ligand (Andersen, 1987; Maloteaux *et al.*, 1988; Mennicken *et al.*, 1992), and tyrosine hydroxylase immunoreactivity.

Early alterations of dopaminergic innervation in the striatum were immediately followed by an increase in peripheral-type benzodiazepine receptors, which are primarily associated with glia (Anholt *et al.*, 1984; Bender *et al.*, 1987; Gehlert *et al.*, 1985) and widely used as markers of reactive microgliosis (Anholt *et al.*, 1984; Benavides *et al.*, 1990, 1991; Myers *et al.*, 1991;

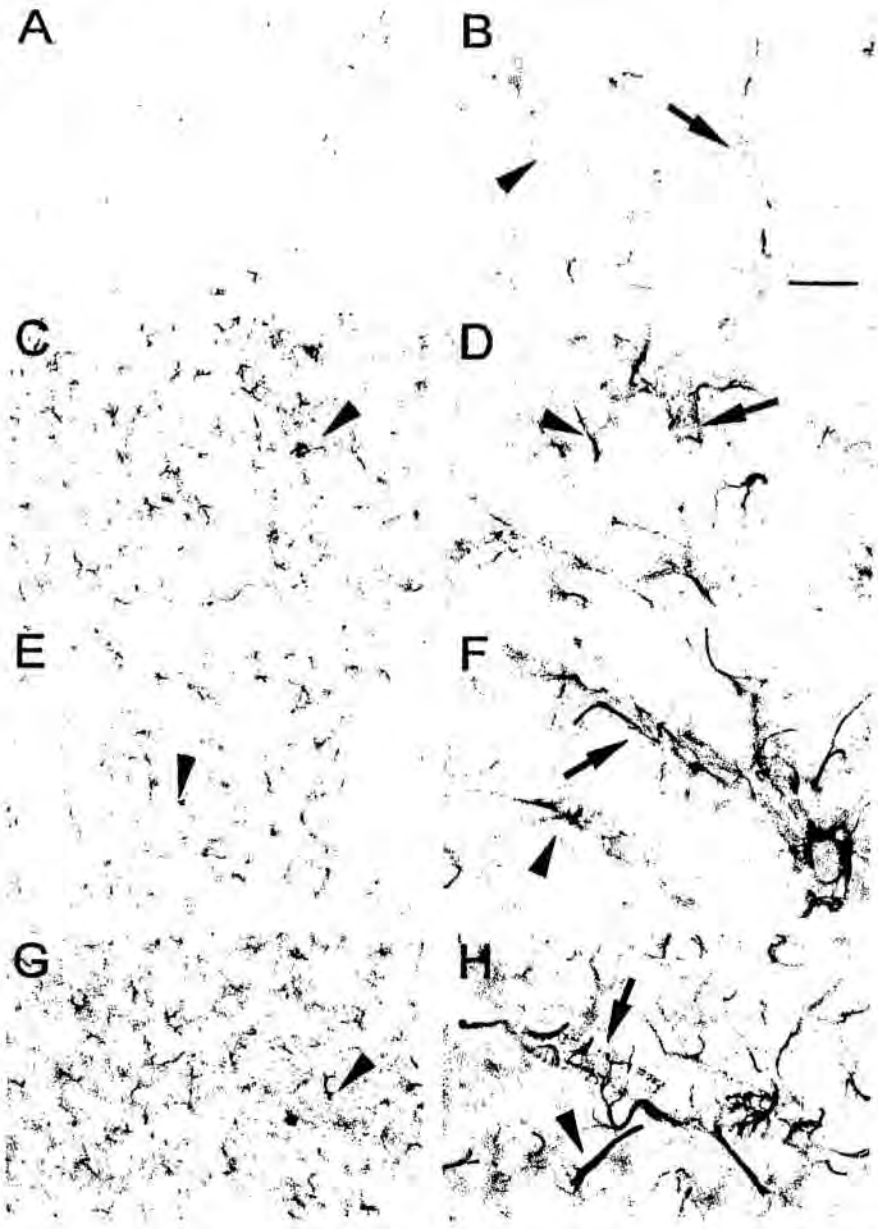

FIG. 6. Immunoreactivity to show GFAP in the contralateral (A, B) and ipsilateral (C–H) striatum at 2 (C, D), 3 (E, F), and 7 (G, H) days after 84 μg MPP^+. Arrowheads point to stained cells, which become more intensely stained in the ipsilateral striatum after MPP^+. Arrows indicate astroglial extensions surrounding blood vessels. Bar: (A, C, E, G) 80 μm; (B, D, F, H) 25 μm.

Stephenson *et al.*, 1995; Kuhlmann and Guilarte, 1997; Camins *et al.*, 1998). Likewise, administration of MPTP to mice does also induce a microglial reaction in the striatum (Kohutnicka *et al.*, 1998; Kurkowska-Jastrzebscka *et al.*, 1999). Injury signals activate resting microglial cells rendering them hyperramified and/or spiny reactive cells, which, if neurons die, acquire an ameboid macrophage-like morphology (Streit *et al.*, 1999). In the present work, resting microglial cells transformed into activated reactive glia in the ipsilateral striatum and they became spiny, which was first detected at 2 days and increased between 3 and 7 days after MPP^+ injection. This morphological shift was accompanied by robust induction of several microglial

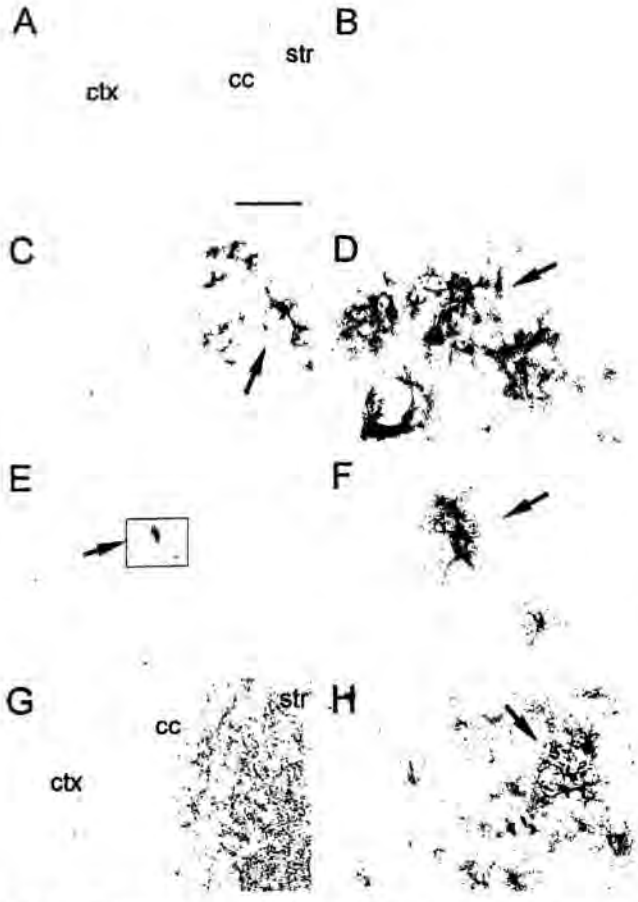

FIG. 7. Induction of the stress protein Hsp27 in the ipsilateral striatum at 2 (C, D), 3 (E, F), and 7 (G, H) days after 84 μg MPP$^+$. No stain was detected in the contralateral striatum (A, B). Arrows show astrocytes immunoreactive to Hsp27. Cortex (ctx), corpus callossum (cc), striatum (str). Bar: (A, C, E) 100 μm; (B, D, F, H) 50 μm; (G) 200 μm.

antigens, such as the CR3 complement receptor (OX42) and MHC class II antigens (OX6). However, ameboid macrophage-like microglial cells, which are phagocytic, were not observed. Indeed, the transformation of resident quiescent microglial cells into reactive microglia within brain parenchyma consists of several stages with different degrees of changes in their phenotype that depend on the severity of injury (Wilson and Molliver, 1994; Kreutzberg, 1996; Streit *et al.*, 1999). Moreover, MPP$^+$ injury to substantia nigra caused an astroglial reaction in the ipsilateral striatum, as shown by increased expression of GFAP and the stress protein Hsp27 in astrocytes. Astrocytic response in the striatum has been reported after i.p. injection of MPTP to mice and cats (Reinhard *et al.*, 1988; Schneider and Denaro, 1988; O'Callaghan *et al.*, 1990b; Kohutnicka *et al.*, 1998).

The secondary glial reaction in the striatum depended on the dose of MPP$^+$ injected in the ipsilateral substantia nigra, as revealed by measurements of peripheral-type benzodiazepine receptor density, and by the intensity and extent of the microglial and astroglial immunoreactions. However, such a dose–response effect was unexpectedly not observed in the assessment of striatal dopamine innervation with [^{3}H]GBR-12935. Indeed, the binding parameter studied here, which was an indicator of dopamine uptake, was already strongly depressed at low doses of MPP$^+$, whereas glial reactivity increased with higher doses. Therefore, it is feasible that the dopaminergic function was lost before any signals able to trigger the glial reactivity were released. Yet, the actual factors that are directly responsible for glial activation in the tissue parenchyma remain unidentified. Experimental studies have shown that microglial cells proliferate inside the brain in response to a circulating cytokine, interferon-γ (Grau *et al.*, 1997). Entry of blood-borne substances into brain tissue can activate astroglia and/or microglia, and serum-derived products induce growth of astrocytes *in vitro* (Giulian *et al.*, 1988). However, the glial reaction that occurs in the striatum after i.p. administration of MPTP in mice does not appear to be mediated by a blood-derived product, since the blood–brain barrier is not altered (O'Callaghan *et al.*, 1990, 1992; Kurkowska-Jastrzebscka *et al.*, 1999). Likewise, our results showed that the integrity of the blood–brain barrier was not affected in the striatum by the injection of MPP$^+$ into the substantia nigra of the rat.

Severe brain injury leading to neuronal cell death induces strong activation of round microglia/macrophages and also astrocytes, which acquire a larger size. However, we found no signs of tissue damage or neural cell death (neither necrotic nor apoptotic) in the striatum. These observations were supported by the use of markers of neurodegeneration and by stereological analysis of neuronal density in the ipsilateral striatum, which were no different from controls at 7 days after MPP$^+$ treatment. However, the stereological method used here did not allow the detection of any neuronal loss affecting less than 6% of neurons, as the coefficient of variation within each treatment group was about 6%. Thus, owing to methodological constraints, we cannot fully discard the possibility that a very low percentage of neurons were lost in the striatum. However, we did not find any evidence of neuronal death using a variety of methodological strategies, including studies of apoptotic and necrotic cell death. Therefore we suggest that glial reactivity in

FIG. 8. Double immunohistochemistry against GFAP (brown) and OX42 (dark blue) in the contralateral (A, D) and ipsilateral (B, C, E–G) striatum at 2 (C, E), 3 (F), and 7 (G) days after 84 μg MPP^+, and 1 day (B) after 28 μg MPP^+. Arrows show reactive microglia and arrowheads point to reactive astrocytes. Both reactive glial cell types colocalized in the same striatal area. Bar: (A–C) 100 μm; (D–G) 30 μm.

the striatum is caused by the damage of nerve cell ends from dopaminergic neurons of the substantia nigra pars compacta, rather than by the loss of striatal neurons. Previous studies with different experimental models of brain injury have shown that axonal degeneration can induce microglial reactivation in remote brain regions (Gehrmann *et al.*, 1991; Fagan & Gage, 1994). Thus, signals other than the local death of neurons can induce gliosis, as previously suggested (Jorgensen *et al.*, 1993). Indeed, IL-6 expression in neurons of transgenic mice causes reactive astrocytosis and increases the number of ramified microglial cells but does not produce neuronal damage (Fattori *et al.*, 1995). Intense neuronal activity, such as that induced by systemic administration of the cholinesterase inhibitor, soman, has also been regarded as a causal agent able to trigger reactivity in neighbouring glia (Zimmer *et al.*, 1997). In this regard, activity induced by cortical spreading depression caused microglial (Gehrmann *et al.*, 1993) and astroglial (Kraig *et al.*, 1991) activation, which were not associated with neuronal damage.

In the present study, reactive spiny microglia colocalized with reactive astroglia in the same striatal areas. However, a different spatial distribution is found in injuries causing striatal cell death, such as focal cerebral ischemia. The latter promotes accumulation of microglia/macrophages within the necrosed core, while large reactive astrocytes are found at the borders of the necrosed area separating the damaged from the non-damaged (Planas *et al.*, 1998; Stoll *et al.*,

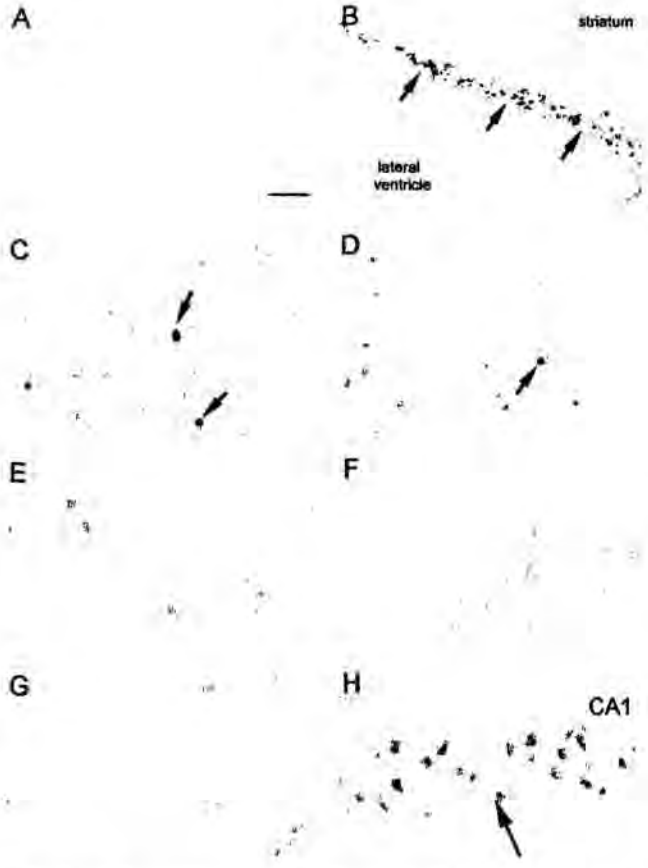

FIG. 9. Cell proliferation (PCNA stain) (A–D) and cell death (tunel) (E–H). (A–D) PCNA immunoreactivity in the contralateral (A, B) and ipsilateral (C, D) striatum at 3 (C) and 7 (D) days after 84 μg MPP^+. Stained cells are normally seen in the subventricular region (B). Very few scattered immunoreactive cells (arrows) are seen in the ipsilateral striatum (C, D). (E–H) Tunel reaction is negative in the ipsilateral striatum after MPP^+ injection in substantia nigra at 1 day (E) and 3 days (F). Positive controls included in the tunel reaction are: ipsilateral subtantia nigra at 3 days after MPP^+ (G) and CA1 layer of the gerbil hippocampus at 4 days after 5 min transient forebrain ischemia (H). Bar: (A, C–H) 50 μm; (B) 100 μm.

1998). Indeed, round macrophage-like microglial cells are thought to release factors exerting some negative effect on astrocytes (Rio *et al.*, 1995). However, in the present study reactive astroglia and microglia had the same spatial distribution, thus indicating that reactive spiny microglia, which have not yet acquired the round macrophage-like morphology, would not necessarily exert a negative control on astrocytes. Microglial and astroglial reactivity in the striatum did not involve extensive cell proliferation as only a few isolated cells had acquired proliferating ability, unlike other brain lesions, such as focal cerebral ischemia that cause strong glial proliferation (Planas *et al.*, 1998).

Several lines of evidence indicate that reactive microglia produce neurotoxic mediators (Wood, 1995; Rogove and Tsirka, 1998). However, other results show that reactive microglial cells can secrete neuroprotective factors suggesting that this reactivity is associated with host defence and neuroprotection (Kreutzberg, 1996; Minghetti and Levi, 1998; Toku *et al.*, 1998; Streit *et al.*, 1999). For instance, activated microglia and macrophages express neurotrophic factors and induce dopaminergic sprouting in the injured striatum (Batchelor *et al.*, 1999). The answer to this controversy might well be that microglia are either neurotrophic or neurotoxic according to the environment and the nature of the activating signals (Zhang and Fedoroff, 1996; Streit *et al.*, 1999). Reactive astrocytes have also been reported to contribute to sprouting through the secretion of neurotrophic factors (Lindsay, 1979; Banker, 1980; Furukawa *et al.*, 1986). It is therefore feasible that the astroglial and microglial reaction detected in the present work was aimed at promoting sprouting and regeneration in the striatum after the degeneration of dopaminergic fibres arising from neurons in the ipsilateral pars compacta of substantia nigra.

In conclusion, degeneration of the nigrostriatal pathway following injection of MPP^+ into substantia nigra induced a pronounced glial reaction in the ipsilateral striatum, although signs of cell death were not apparent in this area. It is suggested that degenerating

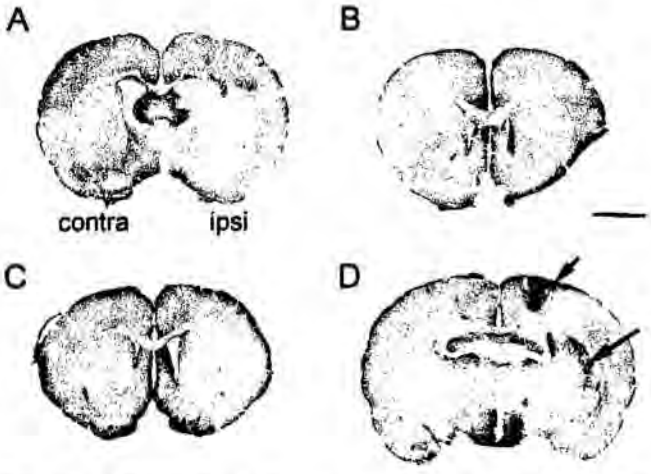

FIG. 10. Albumin stain in coronal brain sections at the level of the striatum at 2 (A), 3 (B), and 7 (C) days after 84 μg MPP^+, or at 7 days after focal cerebral ischemia affecting the hemisphere at the right side of the figure (D). Increased stain is seen in areas where the blood brain barrier has been broken (arrows) after ischemia (D). No alterations are seen in the striatum after MPP^+ (A–D). Bar: 0.3 cm.

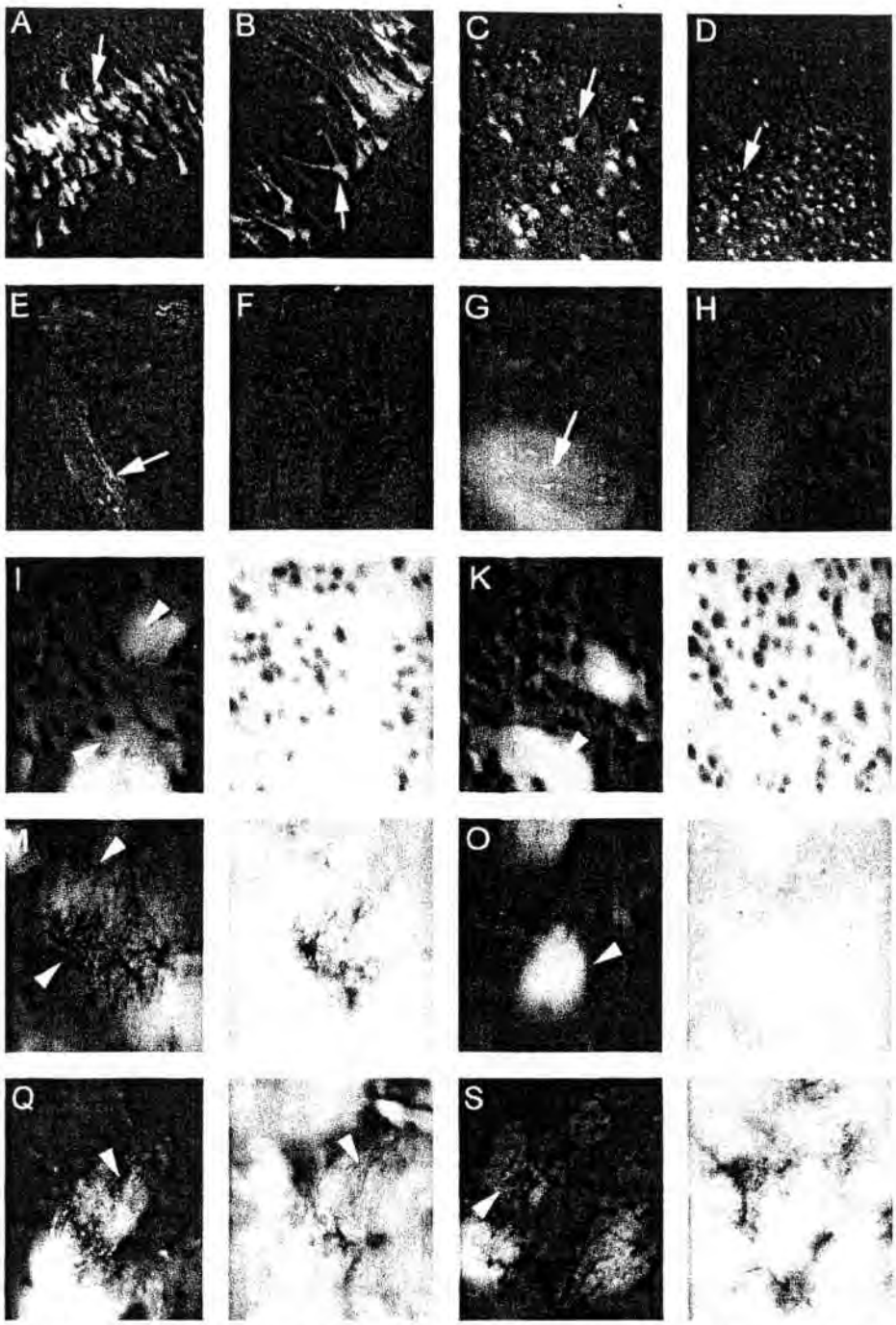

FIG. 11. Fluoro-Jade histochemistry. Positive controls for Fluoro-Jade staining of degenerating neurons (white, arrows) in CA1 (A) and CA3 (B) subfields of the hippocampus at 24 h after kainic acid and in the cortex after focal cerebral ischemia (C, D). Background Fluoro-Jade stain in the contralateral striatum at 3 (F) and 7 (H) days after MPP^+ in the substantia nigra. Increased stain (white, arrows) is seen in fibers within striosomes in the ipsilateral striatum at 3 (E) and 7 (G) days. However, fluorescence is not detected in association with neurons as showed by double-labeling with an antibody against neuron-specific nuclear antigen (black) and Fluoro-Jade (white) in the ipsilateral (I) and contralateral (K) striatum as seen under the fluorescence light. Same fields as seen under the visible light are shown in J and L (arrowheads point to the same areas in striosomes in I and J, and K and L). Double labeling for Hsp27 (black) and Fluoro-Jade (white) is shown in M, O, Q, and S. Corresponding bright fields as seen under visible light are shown in (N, P, R, and T). Contralateral (O, P) and ipsilateral striatum at 2 (M,N,S,T) and 3 (Q, R) days after MPP^+. No Hsp27 is detected in controls (O and P, arrowheads point to the same area in both images). Hsp27-stained cells (arrowheads) appear in association with striosomes in the ipsilateral striatum after MPP^+. Bar: (A–C, G, H, I–T) 30 μm, (D) 60 μm, (E, F) 20 μm.

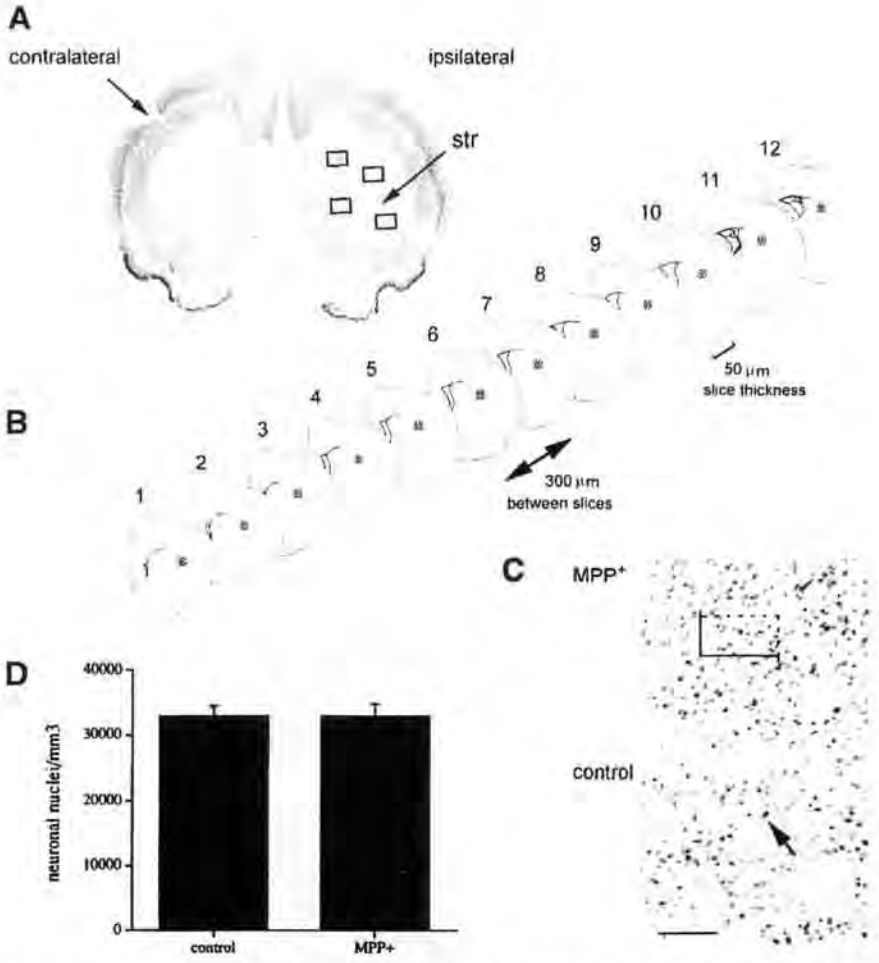

FIG. 12. Inmunostaining against neuron-specific nuclear antigen (Chemicon) (A). Areas used for stereological analysis in the ipsilateral striatum (str) as shown in A (rectangles) were randomly placed. The arrow in the contralateral side shows a cut performed in this hemisphere to identify each side after free-floating inmunohistochemistry. (B) Twelve series of slices (1–12) were used for the analysis. Slice thickness, 50 μm; thickness between slices, 300 μm. (C) Immunohistochemical reaction showing neuron-specific nuclear protein in the striatum, as used for neuronal cell counting. The arrow points to a single neuronal nucleus. The sampling frame is also shown. Bar scale: 100 μm. (D) Estimated neuronal density in the striatum of rats that were MPP^+-treated 7 days earlier (n = 6) was no different from that in controls (n = 6). Bars in the histogram correspond to the standard deviation of the mean.

nerve cell ends, caused by MPP^+ injection in the substantia nigra, release stimuli to the extracellular space that activate resting microglia and astrocytes in the striatum, and that this reaction takes place in absence of neuronal cell death.

ACKNOWLEDGMENTS

This work was supported by grants FIS-97/0490, CICYT- PM98-0195. B. Friguls is recipient of a PhD Fellowship from the University of Barcelona.

REFERENCES

Anholt, R. R. H., Murphy, K. M. M., Mack, G. E., & Snyder, S.H. (1984) Peripheral-type receptors in the central nervous system: localisation to olfactory nerves. *J. Neurosci.* **4**, 593–603.

Andersen, P. H. (1987) Biochemical and pharmacological characterization of [^{3}H] GBR-12935 binding *in vitro* to rat striatal membranes: Labeling of the dopamine uptake complex. *J. Neurochem.* **48**, 1887–1896.

Avendaño, C., & Lagares, A. (1996) A stereological analysis of the numerical distribution of neurons in dorsal root ganglia C4-T2 in adult macaque monkeys. *Somatosens. Mot. Res.* **13**, 59–66.

Banker, G. A. (1980) Trophic interactions between astroglial cells and hippocampal neurons in cultures. *Science* **209**, 809–810.

Batchelor, P. E., Liberatore, G. T., Wong, J. Y. F., Porritt, M. J., Frerichs, F., Donnan, G. A., & Howells, D. W. (1999) Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor. *J. Neurosci.* **19**, 1708–1716.

Benavides, J., Bourdiol, F., Dubois, A., & Scatton, B. (1991) Regional pattern of increased ω_3 (peripheral type benzodiazepine) binding densities in the rat brain induced by systemic injection of kainic acid. *Neurosci. Lett.* **125**, 219–222.

Benavides, J., Fage, D., Carter, C., & Scatton, B. (1990) Peripheral type binding sites are a sensitive indirect index of neuronal damage. *Brain Res.* **421**, 167–172.

Bender, A. S., & Hertz, L. (1987) Pharmacological characteristics of diazepam receptors in neurons and astrocytes in primary cultures. *Brain Res.* **18**, 366–372.

Bowyer, J. F., Peterson, S. L., Rountree, R. L., Tor-Agbidye, J., & Wang, G. J. (1998) Neuronal degeneration in rat forebrain resulting from D-amphetamine-induced convulsions is dependent on seizure severity and age. *Brain Res.* **809**, 77–90.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Camins, A., Gabriel, C., Aguirre, L., Sureda, F. X., Pubill, D., Pallàs, M., Escubedo, E., & Camarasa, J. (1998) U-83836E prevents kainic acid-induced neuronal damage. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **357**, 413–418.

Chiba, K., Trevor, A., & Castagnoli, N., Jr. (1984) Metabolism of neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem. Biophys. Res. Commun.* **120**, 574–578.

Fagan, A. M., & Gage, F. H. (1994) Mechanisms of sprouting in the adult central nervous system: Cellular responses in areas of terminal degeneration and reinnervation in the rat hippocampus. *Neuroscience* **58**, 705–725.

Fattori, E., Lazzaro, D., Musiani, P., Modesti, A., Alonzi, T., & Ciliberto, G. (1995) IL-6 expression in neurons of transgenic mice causes reactive astrocytosis and increase in ramified microglial cells but no neuronal damage. *Eur. J. Neurosci.* **7**, 2441–2449.

Ferrer, I., Olivé, M., Ribera, J., & Planas, A. M. (1996) Naturally occurring (programmed) and radiation-induced apoptosis are associated with selective c-Jun expression in the developing rat brain. *Eur. J. Neurosci.* **8**, 1286–1298.

Ferrer, I., Soriano, M. A., Vidal, A., & Planas, A. M. (1995) Survival of parvalbumin-immunoreactive neurons in the gerbil hippocampus following transient forebrain ischemia does not depend on HSP-70 protein induction. *Brain Res.* **692**, 41–46.

Freyaldenhoven, T. E., Ali, S. F., & Schmued, L. C. (1997) Systemic administration of MPTP induces thalamic neuronal degeneration in mice. *Brain Res.* **759**, 9–17.

Furukawa, S., Furukawa, Y., Satoyoshi, E., & Hayashi, K. (1986) Synthesis and secretion of nerve growth factor by mouse astroglial cells in culture. *Biochem. Biophys. Res. Commun.* **136**, 57–63.

Gehlert, D. R., Yamamura, H. I., & Wamsley, J. K. (1985) Autoradiographic localization of "peripheral-type" benzodiazeine binding sites on the rat brain, heart and kidney. *Naunyn Smiedebergs Arch. Pharmacol.* **328**, 454–460.

Gehrmann, J., Schoen, S. W., & Kreutzberg, G. W. (1991) Lesion of the rat entorhinal cortex leads to a rapid microglial reaction in the dentate gyrus. A light and electron microscopical study. *Acta Neuropathol.* **82**, 442–455.

Gehrmann, J., Bonnekoh, P., Miyazawa, T., Hossmann, K.-A., & Kreutzberg, G. W. (1992) Immunocytochemical study of an early microglial activation in ischemia. *J. Cereb. Blood Flow Metab.* **12**, 257–269.

Gehrmann, J., Mies, G., Bonnekoh, P., Banati, R., Iijima, T., Kreutzberg, G. W., & Hossmann, K.-A. (1993) Microglial reaction in the rat cerebral cortex induced by cortical spreading depression. *Brain Pathol.* **3**, 11–17.

Giulian, D., Woodward, J., Young, D. G., Krebs, J. F., & Lachman, L. B. (1988) Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J. Neurosci.* **8**, 2485–2490.

Graeber, M. B., & Streit, W. J. (1990) Microglia: immune network in the CNS. *Brain Pathol.* **1**, 2–5.

Grau, V., Herbst, B., Van der Meide, P. H., & Steiniger, B. (1997) Activation of microglial and endothelial cells in the rat brain after treatment with interferon-gamma *in vivo*. *Glia* **19**, 181–189.

Gundersen, H. J. G., Bendtsen, T. F., Korbo, L., Marcussen, N., Moller A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sorensen, F. B., Vesterby, A., & West, M. J. (1988a) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* **96**, 379–394.

Gundersen, H. J. G., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sorensen, F. B., Vesterby, A., & West, M. J. (1988b) The new stereological tools: Dissector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* **96**, 857–881.

Heikkila, R. E., Sieber, B. A., Manzino, L., & Sonsalla, P. K. (1989) Some features of the nigrostriatal dopaminergic neurotoxinl methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. *Mol. Chem. Neuropathol.* **10**, 171–183.

Izenwasser, S., Werling, L. L., & Cox, B. M. (1990) Comparison of the effects of cocaine and other inhibitors of dopamine uptake in rat striatum, nucleus accumbens, olfactory tubercle and medial prefrontal cortex. *Brain Res.* **520**, 303–309.

Jakson-Lewis, V., Jacowee, M., Burke, R. E., & Przedborski, S. (1995) Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Neurodegeneration* **4**, 257–269.

Jorgensen, M. B., Finsen, B. R., Jensen, M. B., Castellano, B., Diemer, N. H., & Zimmer, J. (1993) Microglial and astroglial reactions to ischemic and kainic acid-induced lesions of the adult rat hippocampus. *Exp. Neurol.* **120**, 70–88.

Kato, H., Liu, Y., Kogure, K., & Kato, K. (1994) Induction of 27-kDa heat shock protein following cerebral ischemia in a rat model of ischemic tolerance. *Brain Res.* **634**, 235–244.

Kohutnicka, M., Lewandowska, E., Kurkowska-Jastrzebska, Czlonkowski, A., & Czlonkowska, A. (1998) Microglial and astrocytic involvement in a murine model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Immunopharmacology* **39**, 167–180.

Kokaia, Z., Andsberg, G., Yan, Q., & Lindvall, O. (1998) Rapid alterations of BDNF protein levels in the rat brain after focal ischemia: Evidence for increased synthesis and anterograde axonal transport. *Exp. Neurol.* **154**, 289–301.

Kopin, I. J., & Markey, S. P. (1988) MPTP toxicity: Implication for research in Parkinson's disease. *Annu. Rev. Neurosci.* **11**, 81–96.

Kraig, R. P., Dong, L. M., Thisted, R., & Jaeger, C. B. (1991) Spreading depression increases immunohistochemical staining of glial fibrillary acidic protein. *J. Neurosci.* **11**, 2187–2198.

Kreutzberg, G. W. (1996) Microglia: A sensor for pathological events in the CNS. *Trends Neurosci.* **19**, 312–318.

Kuhlmann, A. C., & Guilarte, T. R. (1997) The peripheral benzodiazepine receptor is a sensitive indicator of domic acid neurotoxicity. *Brain Res.* **751**, 281–288.

Kurkowska-Jastrzebska, I., Wronska, A., Kohutnicka, M., Czlonkowski, A., & Czlonkowska A. (1999) The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication in mouse. *Exp. Neurol.* **156**, 50–61.

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

Langston, J. W., & Irwin, I. (1986) MPTP: Currents concepts and controversies. *Clin. Neuropharmacol.* **9**, 485–507.

Lehrmann, E., Christensen, T., Zimmer, J., Diemer, N. H., & Finsen, B. (1997) Microglial and macrophage reactions mark progressive changes and define the penumbra in the rat neocortex and striatum after transient middle cerebral artery occlusion. *J. Comp. Neurol.* **386**, 461–476.

Lindsay, R. M. (1979) Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurons. *Nature* **282**, 80–82.

Lindsay, R. M. (1986) Reactive gliosis. In: *Astrocytes: Cell Biology and Pathology of Astrocytes*, (S. Frderoff, A. Vernadakis, Eds.), Vol 3, pp. 231–262. Academic Press.

Maloteaux, J. M., Vanisberg, M. A., Laterre, C., Javoy-Agid, F., Agid, Y., & Laduron, P. M. (1988) [^{3}H] GBR-12935 binding to dopamine uptake sites: subcellular localization and reduction in Parkinson's disease and progressive supranuclear palsy. *Eur. J. Pharmacol.* **156**, 331–340.

Markey, S. P., Johannessen, J. N., Chiue, C. C., Burns, R. S., & Herkenham, M. A. (1984) Intraneuronal generation of pyridinium metabolite may cause drug-induced parkinsonism. *Nature* **311**, 464–467.

Mennicken, F., Savasta, M., Peretti-Renucci, R., & Feuerstein, C. (1992) Autoradiographic localization of dopamine uptake sites in the rat brain with [^{3}H]-GBR 12935. *J. Neural. Transm. [Gen. Sect.]* **87**, 1–14.

Minghetti, L., & Levi, G. (1998) Microglia as effector cells in brain damage and repair: Focus on prostanoids and nitric oxide. *Prog. Neurobiol.* **54**, 99–125.

Mizuno, Y., Saitoh, T., & Sone, N. (1987) Inhibition of mitochondrial NADH-ubiquinone oxido reductase activity by 1-methyl-4-phenyl-pyridinium ion. *Biochem. Biophys. Res. Commun.* **143**, 294–299.

Myers, R., Manjil, L. G., Cullen, B. M., Price, G. W., Frackowiak, R. S., & Cremer, J. E. (1991) Macrophage and astrocyte populations in relation to [^{3}H]PK 11195 binding in rat cerebral cortex following a local ischaemic lesion. *J. Cereb. Blood Flow Metab.* **11**, 314–322.

Neafsey, E. J., Albores, R., Gearhart, D., Kindel, G., Raikoff, K., Tamayo, F., & Collins, M. A. (1995) Methyl-β-carbolinium analogs of MPP$^+$ cause nigrostriatal toxicity after substantia nigra injections in rats. *Brain Res.* **675**, 279–288.

Nordborg, C., Sokrab, T. E., & Johansson, B. B. (1994) Oedema-related tissue damage after temporary and permanent occlusion of the middle cerebral artery. *Neuropathol. Appl. Neurobiol.* **20**, 56–65.

Norton, W. T., Aquino, D. A., Hozumi, I, Chiu, F. C., & Brosnan, C. F. (1992) Quantitative aspects of reactive gliosis: A review. *Neurochem. Res.* **22**, 877–885.

O'Callaghan, J. P., Miller, D. B., & Reinhard, J. F. (1990a) Characterization of the origins of astrocyte response to injury using the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Res.* **521**, 73–80.

O'Callaghan, J. P., Miller, D. B., & Reinhard, J. F. (1990b) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage of striatal dopaminergic fibers attenuates subsequent astrocyte response to MPTP. *Neurosci. Lett.* **117**, 228–233.

O'Callaghan, J. P. (1988) Neurotypic and gliotypic proteins as biochemical markers of neurotoxicity. *Neurotoxicol. Teratol.* **10**, 445–452.

O'Callaghan, J. P. (1992) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced astrogliosis does not require activation of ornithine decarboxylase. *Neurosci. Lett.* **148**, 10–108.

Pellegrino, L. J., Pellegrino, A. S., & Cushman, A. J. (1967) *A Stereotaxic Atlas of the Rat Brain*, 2nd ed. Plenum Press, New York.

Perry, V. H., & Gordon, S. (1988) Macrophages and microglia in the nervous system. *Trends Neurosci.* **11**, 273–277.

Planas, A. M., Soriano, M. A., Estrada, A., Sanz, O., Martin, F., & Ferrer, I. (1997) The heat shock stress response after brain lesions: induction of 72 kDa heat shock protein (cell types involved, axonal transport, transcriptional regulation) and protein synthesis inhibition. *Prog. Neurobiol.* **51**, 607–636.

Planas, A. M., Justicia, C., Soriano, M. A., & Ferrer, I. (1998) Epidermal growth factor receptor in proliferating reactive glia following transient focal ischemia in the rat brain. *Glia* **23**, 120–129.

Plumier, J. C., Armstrong, J. N., Landry, J., Babity, J. M., Robertson, H. A., & Currie, R. W. (1996) Expression of the 27,000 mol. wt heat shock protein following kainic acid-induced status epilepticus in the rat. *Neuroscience* **75**, 849–856.

Reinhard, J. F. Jr., Miller, D. B., & O'Callaghan, J. P. (1988) The neurotoxicant MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) increases glial fibrillary acidic protein and decreases dopamine levels in the mouse striatum: evidence for glial response to injury. *Neurosci. Lett.* **95**, 246–251.

Reynolds, D. S., & Morton, A. J. (1998) Changes in blood–brain barrier permeability following neurotoxic lesions of rat brain can be visualised with trypan blue. *J. Neurosci. Methods* **79**, 115–121.

Rio, C., Pérez-Cerda, F., Matute, C., & Nieto-Sampedro, M. (1995) Preparation of a monoclonal antibody to a glycidic epitope of the epidermal growth factor receptor that recognizes inhibitor of astrocyte proliferation and reactive microglia. *J. Neurosci. Res.* **40**, 776–786.

Robinson, A. P., White, T. M., & Mason, D. W. (1986) Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognising complement receptor type 3. *Immunology* **57**, 527–531.

Rogove, A. D., & Tsirka, S. E. (1998) Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. *Curr. Biol.* **8**, 19–25.

Santiago, M., Granero, L., Machado, A., & Cano, J. (1995) Complex I inhibitor effect on the nigral and striatal release of dopamine in the presence and absence of nomifesine. *Eur. J. Pharmacol.* **280**, 251–256.

Santiago, M., Machado, A., & Cano, J. (1996) Nigral and striatal comparative study of the neurotoxic action of 1-methyl-4-phenylpyridinium ion: involvement of dopamine uptake system. *J. Neurochem.* **66**, 1182–1190.

Schneider, J. S., & Denaro, F. J. (1988) Astrocytic responses to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-teytahydropyridine (MPTP) in cat and mouse brain. *J. Neuropathol. Exp. Neurol.* **47**, 452–458.

Schmued, L. C., Albertson, C., & Slikker, W., Jr. (1997) Fluoro-Jade: A novel fluorochrome for the sensitive and reliable histochemical localisation of neuronal degeneration. *Brain Res.* **751**, 37–46.

Soriano, M. A., Planas, A. M., Rodríguez-Farré, E., & Ferrer, I. (1994) Early 72-kDa heat shock protein induction in microglial cells following focal ischemia in the rat brain. *Neurosci. Lett.* **182**, 205–207.

Soriano, M. A., Justicia, C., Ferrer, I., Rodríguez-Farré, E., & Planas, A. M. (1997) Striatal infarction in the rat causes a transient reduction of tyrosine hydroxylase immunoreactivity in the ipsilateral substantia nigra. *Neurobiol. Dis.* **4**, 376–385.

Stephenson, D. T., Schober, D. A., Smalstig, E. B., Mincy, R. E., Gehlert, D. R., & Clemens, J. A. (1995) Peripheral benzodiazepine receptors are colocalized with activated microglia following transient global forebrain ischemia in the rat. *J. Neurosci.* **15**, 5263–5274.

Sterio, D. C. (1984) The Unbiased estimation of number and sizes of arbitrary particles using the dissector. *J. Microsc.* **134**, 127–136.

Stoll, G., Jander, S., & Schroeter, M. (1998) Inflammation and glial responses in ischemic brain lesions. *Prog. Neurobiol.* **56**, 149–171.

Streit, W. J., Graeber, M. B., & Kreutzberg, G. W. (1988) Functional plasticity of microglia: A review. *Glia* **1**, 301–307.

Streit, W. J., Walter, S. A., & Pennell, N. A. (1999) Reactive microgliosis. *Prog. Neurobiol.* **57**, 563–581.

Sun, C. J., Johannessen, J. N., Gessner, W., Namura, I., Singhaniyom, W., Brossi, A., & Chiueh, C. C. (1988) Neurotoxic damage to the nigrostriatal system in rats following intranigral administration of $MPDP^+$ and MPP^+. *J. Neural. Transm.* **74**, 75–86.

Tatton, N. A., & Kish, S. J. (1997) In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labeling and acridine orange staining. *Neuroscience* **77**, 1037–1048.

Ter Horst, G. J., Knigge, M. F., & Van der Wal, A. (1992) Neurochemical lesioning in the rat brain with iontophoretic injection of the 1-methyl-4-phenylpyridinium ion (MPP^+). *Neurosci. Lett.* **141**, 203–207.

Toku, K., Tanaka, J., Yano, H., Desaki, J., Zhang, B., Yang, L., Ishihara, K., Sakanaka, M., & Maeda, N. (1998) Microglial cells prevent nitric oxide-induced neuronal apoptosis *in vitro*.

West, M. J., Østergaard, K., Andreassen, O. A., & Finsen, B. (1996) Estimation of the number of somatostain neurons in the striatum: An *in situ* hybridization study using the optical fractionator method. *J. Comp. Neurol.* **370**, 11–22.

Wilson, M. A., & Molliver, M. E. (1994) Microglial response to degeneration of serotonergic axon terminals. *Glia* **11**, 18–34.

Wood, P. L. (1995) Microglia as a unique cellular target in the treatment of stroke: Potential neurotoxic mediators produced by activated microglia. *Neurol. Res.* **17**, 242–248.

Zhang, S. C., & Fedoroff, S. (1996) Neuron-microglia interactions in vitro. *Acta Neuropathol.* (Berl) **91**, 385–395.

Zimmer, L. A., Ennis, M., & Shipley, M. T. (1997) Soman-induced seizures rapidly activate astrocytes and microglia in discrete brain regions. *J. Comp. Neurol.* **378**, 482–492.

BRAIN-DERIVED NEUROTROPHIC FACTOR, NEUROTROPHIN-3 AND NEUROTROPHIN-4/5 PREVENT THE DEATH OF STRIATAL PROJECTION NEURONS IN A RODENT MODEL OF HUNTINGTON'S DISEASE

Publicat a la revista Journal of Neurochemistry **75**, 2190-2199 (2000)

Journal of Neurochemistry
Lippincott Williams & Wilkins, Inc., Philadelphia

Brain-Derived Neurotrophic Factor, Neurotrophin-3, and Neurotrophin-4/5 Prevent the Death of Striatal Projection Neurons in a Rodent Model of Huntington's Disease

*†Esther Pérez-Navarro, *‡Anna M. Canudas, †Peter Åkerud, *Jordi Alberch, and †Ernest Arenas

*Departament de Biologia Cel.lular i Anatomia Patològica, Facultat de Medicina, IDIBAPS, Universitat de Barcelona; ‡Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari Pedralbes, Barcelona, Spain; and †Department of Medical Biochemistry and Biophysics, Laboratory of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden

Abstract: Intrastriatal injection of quinolinate has been proven to be a very useful animal model to study the pathogenesis and treatment of Huntington's disease. To determine whether growth factors of the neurotrophin family are able to prevent the degeneration of striatal projection neurons, cell lines expressing brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), or neurotrophin-4/5 (NT-4/5) were grafted in the adult rat striatum before quinolinate injection. Three days after lesioning, ongoing cell death was assessed by in situ detection of DNA fragmentation. In animals grafted with the control cell line, quinolinate injection induced a gradual cell loss that was differentially prevented by intrastriatal grafting of BDNF-, NT-3-, or NT-4/5-secreting cells. Seven days after lesioning, we characterized striatal projection neurons that were protected by neurotrophins. Quinolinate injection, alone or in combination with the control cell line, induced a selective loss of striatal projection neurons. Grafting of a BDNF-secreting cell line prevented the loss of all types of striatal projection neurons analyzed. Glutamic acid decarboxylase 67-, preproenkephalin-, and preprotachykinin A- but not prodynorphin-expressing neurons were protected by grafting of NT-3- or NT-4/5-secreting cells but with less efficiency than the BDNF-secreting cells. Our findings show that neurotrophins are able to promote the survival of striatal projection neurons in vivo and suggest that BDNF might be beneficial for the treatment of striatonigral degenerative disorders, including Huntington's disease. **Key Words:** Rat striatum—Quinolinate—Grafting—Survival.
J. Neurochem. **75,** 2190–2199 (2000).

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine repeat in the huntingtin gene (MacDonald and Gusella, 1996; Mangiarini et al., 1996). Its predominant pathological feature is a massive and progressive degeneration of striatal output neurons without substantial loss of striatal interneurons and afferents (for review, see DiFiglia, 1990). Intrastriatal injection of quinolinate (QUIN), an NMDA receptor agonist, replicates many neurochemical, histological, and behavioral features of HD (Beal et al., 1986; DiFiglia, 1990). Striatal projection neurons containing enkephalin are affected to a greater extent than substance P-containing neurons, and those neurons surviving the lesion express reduced levels of their mRNAs both in HD (Reiner et al., 1988; Richfield et al., 1995) and after QUIN injections (Pérez-Navarro et al., 1999*a,b*). Excitotoxicity and apoptosis have been suggested to be involved in the degeneration of neurons in HD (Thomas et al., 1995; Petersén et al., 1999) and after QUIN injection (Ferrer et al., 1995; Portera-Cailliau et al., 1995; Hughes et al., 1996). Furthermore, intrastriatal QUIN injections have been found to induce huntingtin mRNA (Carlock et al., 1995) and protein (Tatter et al., 1995), providing a possible link between the QUIN model and HD.

Members of the neurotrophin family have been suggested as therapeutic candidates for neurodegenerative disorders because they promote neuronal survival in different lesion models (for review, see Hefti, 1994; Connor and Dragunow, 1998). Neurotrophins show high-affinity interactions with the tyrosine kinase receptors TrkA,

Received February 17, 2000; revised manuscript received June 28, 2000; accepted July 8, 2000.

Address correspondence and reprint requests to Dr. E. Arenas at Department of Medical Biochemistry and Biophysics, Laboratory of Molecular Neurobiology, Karolinska Institute, Stockholm S-17177, Sweden. E-mail: Ernest@cajal.mbb.ki.se

Abbreviations used: BDNF, brain-derived neurotrophic factor; DYN, prodynorphin; F3A-MT, F3A-NT3, F3A-NT4/5, and F3N-BDNF, mock-transfected, neurotrophin-3-transfected, neurotrophin-4/5-transfected, and brain-derived neurotrophic factor-transfected, respectively, Fischer-344 rat 3T3 fibroblasts; GAD, glutamic acid decarboxylase 67; HD, Huntington's disease; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; PPE, preproenkephalin; PPTA, preprotachykinin; QUIN, quinolinate; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase-mediated UTP nick end-labeling.

TrkB, and TrkC. These receptors mediate neurotrophin signaling, a process that involves tyrosine phosphorylation. Nerve growth factor binds to TrkA, and brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) bind to TrkB, whereas neurotrophin-3 (NT-3) binds preferentially to TrkC and to a lesser extent to TrkA and TrkB (for review, see Barbacid, 1994). In addition, the p75 neurotrophin receptor is able to bind all neurotrophins and could represent an important switch between life and death signaling (Carter and Lewin, 1997). During development, both p75 and Trk receptors are expressed in the striatum (Ernfors et al., 1992), and adult striatal projection neurons express both TrkB and TrkC mRNAs (Merlio et al., 1992). In agreement with the presence of neurotrophin receptors on striatal projection neurons, BDNF, NT-3, and NT-4/5 have been found to promote the survival of striatal projection neurons in vitro (Ardelt et al., 1994; Widmer and Hefti, 1994; Nakao et al., 1995*a*,*b*; Ventimiglia et al., 1995). In contrast, the effects of these neurotrophins on striatal projection neurons in vivo remain unclear. Although some reports did not find any neuroprotective effect of BDNF or NT-3 (Frim et al., 1993; Anderson et al., 1996), other studies have found that BDNF and NT-4/5 partially protect striatal projection neurons from excitotoxic lesions (Martínez-Serrano and Björklund, 1996; Alexi et al., 1997) and that BDNF, NT-3, and NT-4/5 differentially regulate the phenotype of striatal projection neurons (Pérez-Navarro et al., 1999*a*).

In the present study, we have examined whether BDNF, NT-3, and/or NT-4/5 may prevent the death and promote the survival of different populations of striatal projection neurons in a QUIN model of HD. Stable cell lines secreting high levels of recombinant BDNF, NT-3, or NT-4/5 were implanted in adult rat striatum before QUIN injection. We show that the three neurotrophins differentially prevent the death of different subsets of striatal projection neurons in vivo and that BDNF is the most efficient survival factor.

MATERIALS AND METHODS

Animal subjects

Adult male Fischer-344 rats (weighing 200–250 g) were used in this study. After surgery, animals were housed separately with food and water ad libitum in a colony room maintained at a constant temperature (19–22°C) and humidity (40–50%) on a 12:12 h light/dark cycle. Animal treatment was performed according to Directive 86/609/EEC of the European Commission.

Cell grafting and QUIN lesion

BDNF- (F3N-BDNF), NT-3- (F3A-NT3), or NT-4/5- (F3A-NT4/5) transfected Fischer-344 rat 3T3 fibroblasts have been described previously and shown to produce >100 ng of each neurotrophin/10^6 cells per day, in vitro (Arenas and Persson, 1994; Neveu and Arenas, 1996). Mock-transfected Fischer-344 rat 3T3 fibroblasts (F3A-MT) were used as a control (Arenas and Persson, 1994). All cell lines were grown in Dulbecco's modified essential medium supplemented with 10% fetal calf serum, 1 mg/ml penicillin–streptomycin, 1 mg/ml glutamine, and 200 μg/ml G-418 (37°C, 5% CO_2). For grafting, cells in active growth phase were washed and collected in serum-free medium at a concentration of 2.5×10^5 cells/μl as described (Arenas and Persson, 1994). A microinjection cannula was implanted into the left striatum, and 3 μl containing 7.5×10^5 cells was injected (1 μl/min) at the following coordinates: 1.8 mm cranial to bregma, 3.2 mm lateral to the midline. Two microinjections of 34 nmol of QUIN each were performed 24 h later, as described previously (Pérez-Navarro et al., 1996, 1999*a*,*b*), at the following coordinates: (a) 2.2 mm cranial to bregma, 2.9 mm lateral to the midline; and (b) 0.8 mm cranial to bregma, 3.5 mm lateral to the midline. In all cases, the injection was performed at 5.2 mm under the dural surface with the incisor bar at 5 mm above the interaural line.

BDNF and NT-3 ELISA

Seven days after QUIN injection in F3A-MT-, F3N-BDNF-, or F3A-NT3-grafted striata, rats were killed by decapitation. Brains were removed, and neostriata were quickly dissected out, frozen on dry ice, and stored at −80°C. Samples were analyzed using a BDNF or NT-3 ELISA kit according to the recommendations of the manufacturer (Promega). In brief, striata were lysed in 1 ml of lysis buffer [137 m*M* NaCl, 20 m*M* Tris, 1% (octylphenoxy)polyethoxyethanol (IGEPAL), 10% glycerol, 10 m*M* NAF, 2 m*M* sodium vanadate, and proteinase inhibitor cocktail tablets]. A volume of 25 μl of the sample was diluted in 75 μl of sample buffer and incubated in a plate coated with a BDNF or NT-3 antibody. Two dilutions (1:100 and 1:10,000) of each sample were analyzed. Standard curves of pure BDNF or NT-3 protein, provided by the kit, were used to quantify the production of BDNF or NT-3.

Immunohistochemistry

Seven days after QUIN injection in F3A-MT- or F3N-BDNF-grafted striata, animals were killed by decapitation. Brains were removed, frozen on dry ice, and stored at −70°C. Cryostat-cut horizontal sections (14 μm) through the whole striatum were serially collected on silane-coated slides. Sections were incubated overnight at 4°C with a polyclonal antibody against phosphorylated Trk (pY-490; 1:10; a generous gift from Dr. Rosalind A. Segal) alone or in the presence of 1 μ*M* competing phosphopeptide (686) in Tris-buffered saline with 1 m*M* sodium orthovanadate, 5% goat serum, and 0.5% NP-40. After washing, the slides were incubated for 1 h at room temperature with a secondary tetramethylrhodamine B isothiocyanate-labeled goat anti-rabbit antibody (1:50, in Tris-buffered saline with 1 m*M* sodium orthovanadate), washed, and mounted in Tris-buffered saline with glycerol containing *p*-phenylenediamine. Optical density was quantified using a Quantimed 570 Image processing and Analysis System linked to a Microphot-FXA microscope (Nikon, Garden City, NY, U.S.A.) by an interfacing CCD videocamera. Measurements were performed in horizontal sections through the maximal diameter of the graft. Readings of optical density were obtained from the border of the graft, to the end of the striatum, in a sagittal and caudal direction. Measures of background staining, obtained from sections preincubated with the competing phosphopeptide, were subtracted from the values obtained for the noncompeted pY-490 staining in adjacent sections and expressed as net optical units. Three sections per animal, in three animals per condition, were analyzed.

In situ detection of DNA fragmentation

Three days after intrastriatal QUIN injection, animals grafted with F3A-MT, F3N-BDNF, F3A-NT3, or F3A-NT4/5

were deeply anesthetized and immediately perfused transcardially with saline, followed by 4% paraformaldehyde/phosphate-buffered saline (0.1 *M*, pH 7.4). Brains were removed and postfixed for 1–2 h in the same solution, cryoprotected by immersion in 30% sucrose in phosphate-buffered saline, and then frozen in dry ice-cooled isopentane. Cryostat-cut horizontal sections (14 μm) through the whole striatum were serially collected on silane-coated slides. DNA fragmentation was histologically examined using the in situ Apoptosis Detection System, Fluorescein (Promega). Sections were stained according to the manufacturer's recommendations. In brief, sections were immersed in cool ethanol/acetic acid (2:1 vol/vol) for 5 min and washed in two changes of phosphate-buffered saline at room temperature. Sections were treated with proteinase K (20 μg/ml) during 10 min and postfixed in 4% paraformaldehyde in phosphate-buffered saline for 5 min. Sections were incubated with the equilibration buffer for 5 min at room temperature and then with fluorescein-12-dUTP and terminal deoxynucleotidyl transferase (TdT). After a 1-h incubation at 37°C, sections were washed with 2× saline–sodium citrate for 15 min, and three times with phosphate-buffered saline for 10 min and mounted with Mowiol. As a negative control, adjacent sections were processed following the standard procedure, except that TdT was substituted by water.

In situ hybridization

Seven days after QUIN injection, animals were killed by decapitation. Brains were removed, frozen on dry ice, and stored at −70°C. Cryostat-cut horizontal sections (14 μm) through the whole striatum were serially collected on silane-coated slides, fixed with 4% paraformaldehyde in phosphate-buffered saline, dehydrated in graded ethanol solutions, treated with chloroform, and air-dried. Adjacent sections were processed for in situ hybridization with oligonucleotide probes for rat glutamic acid decarboxylase 67 (GAD), preproenkephalin (PPE), preprotachykinin A (PPTA), or prodynorphin (DYN) as previously described (Arenas et al., 1996; Pérez-Navarro et al., 1999*b*). The slides were exposed to β-Max x-ray film for 20 days, dipped in NTB-2 photoemulsion (diluted 1:1 in water) for 40 days at 4°C, developed in D-19, fixed, and lightly counterstained with cresyl violet before analysis.

Image analysis and morphometry

All morphometry and cell counting were performed in a blind coded fashion. Area of the lesion and cell counting measurements were performed using PC-Image analysis (Foster Findlay) on a computer attached to an Olympus microscope as previously described (Pérez-Navarro et al., 1999*a*,*b*).

For the lesion size estimations, consecutive sections (average of 20–22 sections per animal and probe) were visualized on a computer, and the border of the lesion was outlined. The volume of the lesion was estimated by multiplying the sum of all the sectional areas (in square micrometers) by the distance between successive sections (98 μm), as previously described (Coggeshall, 1992).

PPE-, GAD-, PPTA-, and DYN-positive neurons were counted in a region caudal to the graft, in four different fields (130 × 86 μm, each). The first field analyzed was the one where the first labeled neurons were observed. This field was located at different distances from the border of the graft depending on the in situ probe used (Pérez-Navarro et al., 1999*a*). The other three regions were successively located 325 μm away from the previous one and aligned with the center of the graft, as previously described (Pérez-Navarro et al., 1999*a*,*b*). Cell counts were converted to the number of cells per square millimeter by dividing by the area corresponding to each field examined (0.0112 mm^2). TdT-mediated UTP nick end-labeling (TUNEL)-labeled cells were also quantified in a region caudal to the graft by direct counting of fluorescein-stained nuclei. Three adjacent fields (305 × 205 μm, each) beginning at the border of the graft and aligned with the center of the graft were counted.

All striatal neuronal populations and TUNEL-labeled cells were counted in 10 sections per animal, separated by 150 μm, and in four animals per condition (F3A-MT, F3N-BDNF, F3A-NT3, or F3A-NT4/5 grafting with or without QUIN injection).

Materials

All the reagents used for cultures were from GIBCO. QUIN and IGEPAL were obtained from Sigma Chemical Co. The ELISA kit and in situ Apoptosis Detection System, Fluorescein were from Promega. β-Max x-ray film was from Amersham. NTB-2 photoemulsion and D-19 developer were from Kodak. Tetramethylrhodamine B isothiocyanate-labeled goat anti-rabbit antibody was from Jackson, Mowiol was purchased from Calbiochem, and proteinase inhibitor cocktail tablets were from Boehringer Mannheim.

RESULTS

Characterization of graft size and neurotrophin production by grafts

To control the growth rate of the different cell lines, the size of the grafts was measured 8 days after grafting, in all the sections used to count the number of striatal neurons. The mean radius (in μm) of the grafts in nonlesioned animals was similar for all the cell lines used (Fig. 1A). In QUIN-lesioned animals the size of the grafts was also similar for all the cell lines but slightly larger than in nonlesioned animals (Fig. 1A).

The levels of BDNF or NT-3 secreted by the cell lines were determined by ELISA, 7 days after QUIN injection. Our results show that grafting of the F3N-BDNF or the F3A-NT3 cell lines increased the levels of the corresponding neurotrophin in the striatum by 17 and 10 times, respectively, compared with striata receiving the control graft plus QUIN injection (Fig. 1B and C). The actual dose of neurotrophins delivered by the cells cannot be calculated per unit of time from our measures in vivo because the kinetics of internalization and metabolism of neurotrophins have not been accounted for. However, our results clearly show that for a certain given time, 7 days after QUIN injection, both cell lines were able to increase by at least one order of magnitude the levels of protein available in the striatum.

Grafting of BDNF-secreting cell line induces TrkB phosphorylation

We first set out to determine the range and the extent to which neurotrophins secreted by the cell lines were able to activate their receptors within the striatum. For that purpose, we examined TrkB phosphorylation in response to intrastriatal grafting of BDNF-secreting cell line because BDNF has been reported to be the neurotrophin with the lowest intraparenchymal diffusion (for review, see Mufson et al., 1999). Seven days after QUIN injection in F3A-MT- or F3N-BDNF-grafted striata, sec-

A

	Mean radius (μm)	
	Non-lesioned	Lesioned
F3A-MT	640±75	795±50
F3N-BDNF	633±85	860±48
F3A-NT3	614±51	799±42
F3A-NT4	513±68	724±61

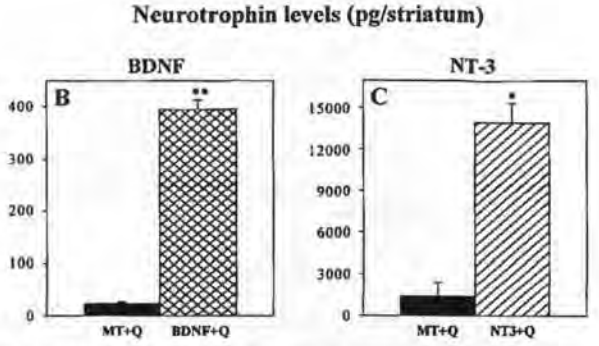

FIG. 1. Characterization of graft size and neurotrophin production. **A:** Radius (in μm) of the grafts 8 days after grafting (nonlesioned animals) or 7 days after intrastriatal QUIN injection. Data are mean ± SEM values (n = 4 animals per condition). All grafts showed similar size but were slightly larger in QUIN-lesioned striata. **B** and **C:** Content of (B) BDNF or (C) NT-3, measured by ELISA, in striata of adult rats receiving grafts of F3A-MT (MT), F3N-BDNF (BDNF), or F3A-NT3 (NT3) cell lines and QUIN (Q) injections. $^{*}p < 0.01$, $^{**}p < 0.001$ compared with MT + Q-injected striata by Student's *t* test for unpaired data.

tions were processed by immunohistochemistry with an antibody against phosphorylated TrkB [pY-490 (Segal et al., 1996)]. Optical densities were measured at different distances from the border of the graft. As shown in Fig. 2A, we observed low levels of TrkB phosphorylation in the vicinity of the lesion and a gradual increase in the staining of phospho-TrkB at increasing distances from the border of the grafts. In animals grafted with F3A-MT cells and injected with QUIN, phospho-TrkB levels were very low from the border of the graft up to a distance of 300 μm, where neurons were not observed by in situ hybridization (Pérez-Navarro et al., 1999*a*). In this condition, phospho-TrkB levels reached levels comparable to those found in the intact striatum at a distance of 450 μm from the border of the graft and then mildly increased to reach a plateau at 600 μm. In contrast, in the F3N-BDNF-grafted striata, phospho-TrkB levels progressively increased and reached maximal levels at 900–1,050 μm from the border of the graft (Fig. 2A). Beyond 1,200 μm from the border of the graft, phospho-TrkB levels declined in both BDNF- and control-grafted striata. However, BDNF grafts maintained higher levels of phospho-TrkB than control grafts 300 μm to up to 1,500 μm from the border of the graft (Fig. 2A). Interestingly we found both strongly immunolabeled neuronal somas in the striatum (Fig. 2B) and sporadic immunoreactive fibers around the F3N-BDNF graft (Fig. 2C), indicating that TrkB phosphorylation could result from direct activation of TrkB receptors on neuronal somas and/or retrograde transport of phosphorylated TrkB to the cell bodies.

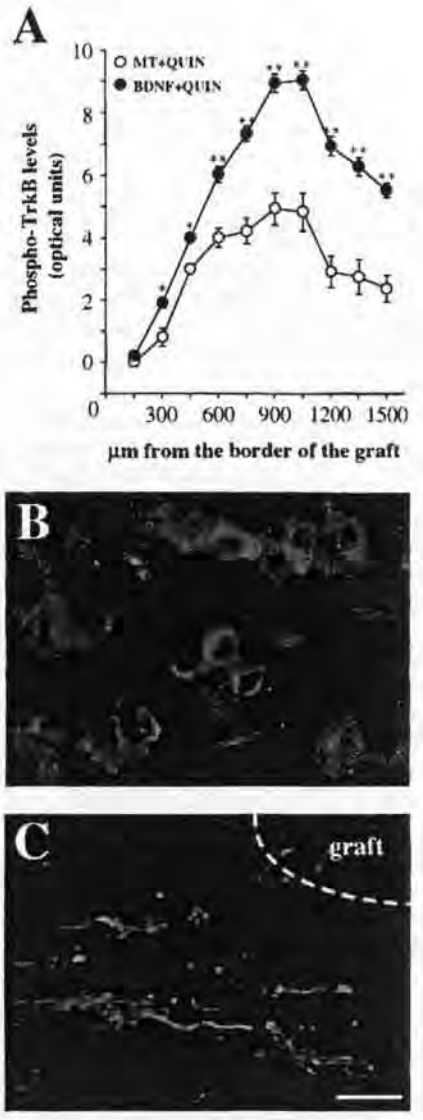

FIG. 2. Intrastriatal grafting of the BDNF-secreting cell line induced TrkB phosphorylation. **A:** Optical density was measured at different distances from the border of the graft. Data are mean ± SEM (bars) values (n = 4 animals per condition). Clear higher increases in phospho-TrkB levels were detected from a distance of 300 μm in lesioned animals grafted with the BDNF cell line (●) compared with lesioned animals grafted with the control cells (MT; ○). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with MT plus QUIN-injected striata by Student's *t* test for unpaired data. **B:** Photomicrograph shows neurons immunostained with an anti-phospho-TrkB antibody at 600 μm from the border of the graft in animals grafted with F3N-BDNF and injected with QUIN. **C:** Neuronal fibers positive for anti-phospho-TrkB were sporadically observed in the vicinity of the graft. Bar = 20 μm.

Neurotrophins prevent striatal cell death induced by QUIN

Striatal cell death was studied 3 days after QUIN injection. We chose this time point because apoptotic profiles reach maximal levels at 48–72 h after intrastriatal QUIN injection (Hughes et al., 1996). DNA fragmentation was histologically examined using the TUNEL technique. In the absence of TdT, no nuclei were stained, confirming the specificity of the labeling. Labeled nuclei were only observed in the QUIN-injected striata, and no signal was detected in other brain areas.

Stained nuclei were counted in three adjacent fields, beginning at the border of the graft and located in the region caudal to the graft. In animals grafted with the control cell line, QUIN injection induced a gradual loss of striatal neurons, as shown by the decrease in the number of labeled nuclei (Fig. 3E). Intrastriatal grafting of F3N-BDNF, F3A-NT3, or F3A-NT4/5 cell lines differentially prevented QUIN-induced cell death in all the striatal regions examined (Fig. 3E). Data obtained from these animals indicated that most of the damage induced by QUIN was limited to the area between the graft and 1 mm away. To evaluate the total effect of neurotrophins, the average density of TUNEL-labeled nuclei was calculated in the first millimeter surrounding the graft. We found that the F3N-BDNF cell line, which reduced the number of TUNEL-labeled nuclei by 57%, was the most efficient at preventing cell death. F3A-NT3 and F3A-NT4/5 grafting showed lower efficiency and prevented striatal cell death by 37 and 31%, respectively.

Effect of neurotrophins on lesion size induced by QUIN

As neurotrophins prevented QUIN-induced cell death, we next examined which population of striatal projection neurons was protected by these factors. In situ hybridization for different markers (GAD, PPE, PPTA, and DYN), characteristic of striatal projection neurons, was performed. Intrastriatal injection of QUIN alone induced a lesion with a size (striatal volume without in situ labeled neurons) that was not modified by grafting of the control cell line (F3A-MT; Fig. 4). In agreement with previous results (Pérez-Navarro et al., 1999*a*,*b*), GAD- and PPE-positive neurons were more affected than PPTA- and DYN-positive cells (Fig. 4). Grafting of neurotrophin-secreting cell lines reduced the lesion size of neurons positive for GAD (Fig. 4A), PPE (Fig. 4B), and PPTA (Fig. 4C), showing that BDNF and NT-3 were more efficient than NT-4/5. In contrast, in the same animals, none of the grafts reduced the volume of the lesion for DYN-positive neurons (Fig. 4D).

Grafting of the control cell line did not prevent loss of striatal projection neurons induced by QUIN

The number of surviving neurons was examined by in situ hybridization after implantation of F3A-MT, F3N-BDNF, F3A-NT3, or F3A-NT4/5 in lesioned and nonlesioned animals. Positive neurons were counted in four different fields located in the region caudal to the graft

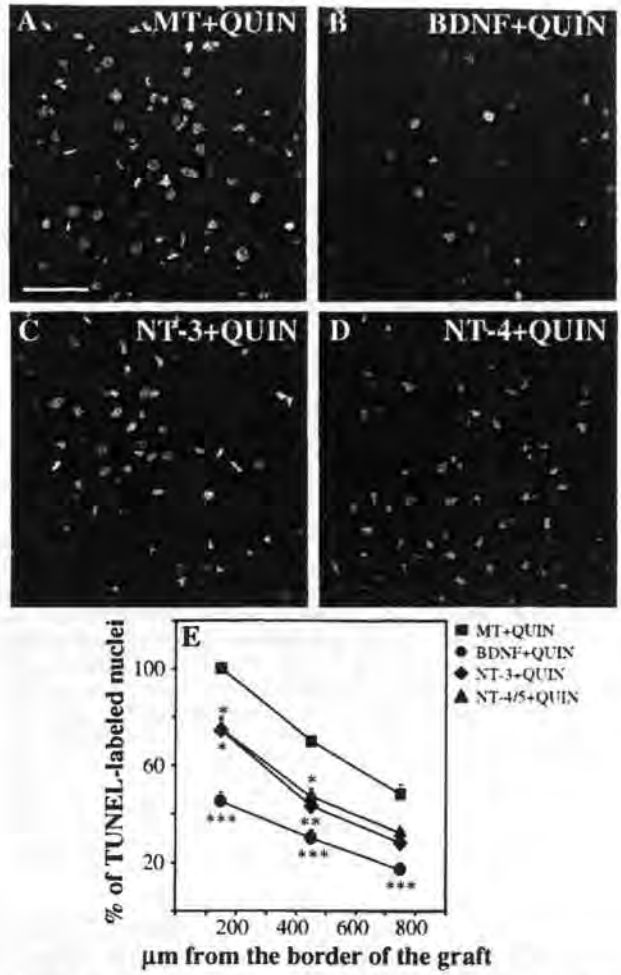

FIG. 3. Neurotrophins prevent DNA fragmentation induced by intrastriatal QUIN injection. Striata were examined 3 days after QUIN injection in animals grafted with control or BDNF-, NT-3-, or NT-4/5-secreting cell lines. Images show confocal laser scanning photomicrographs of TUNEL-stained nuclei at the level of the first region (located at the border of the graft). Grafting of (**B**) BDNF-, (**C**) NT-3-, or (**D**) NT-4/5-secreting cells decreased the number of labeled nuclei compared with the control cell line (**A**). Bar in A = 40 μm. **E:** Mean ± SEM (bars) number of labeled nuclei (n = 4 animals per condition). The number of nuclei in every region and condition was standardized to the values obtained for the first region in animals grafted with the control cell line (values in this region were considered as 100%, as this region was the most affected by QUIN injection). MT + QUIN, mock-transfected control cell line-grafted striata plus QUIN injection (■); BDNF + QUIN, striata grafted with BDNF-secreting cell line plus QUIN injection (●); NT-3 + QUIN, striata grafted with NT-3-secreting cell line plus QUIN injection (◆); NT-4/5 + QUIN, striata grafted with NT-4/5-secreting cell line plus QUIN injection (▲). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with MT + QUIN-injected striata by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test.

(see Materials and Methods). In the nonlesioned striatum, grafting of the different cell lines did not produce changes in the total number of striatal projection neurons compared with the contralateral, noninjected side (data not shown). QUIN injection alone induced a gradual loss

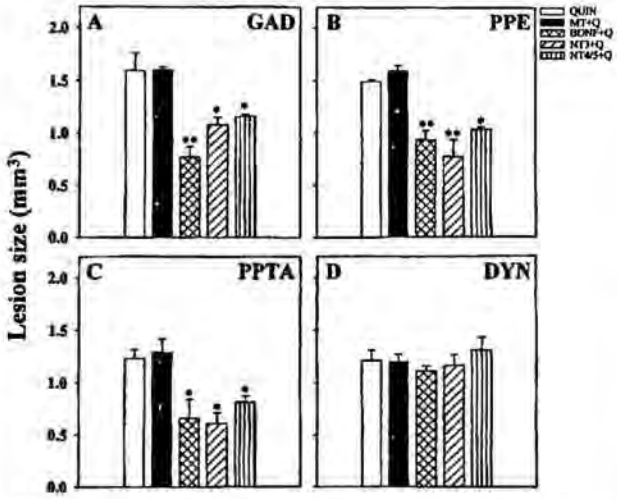

FIG. 4. Neurotrophins differentially reduced the size of the lesion induced by QUIN injections. The volume of the lesion was measured for each striatal neuronal population in sections hybridized with (**A**) GAD, (**B**) PPE, (**C**) PPTA, and (**D**) DYN antisense probes. Data are mean ± SEM (bars) values (n = 4 animals per condition). Grafting of the control cell line (MT+Q) did not modify the volume of the lesion induced by QUIN injection alone. In contrast, intrastriatal grafting of F3N-BDNF (BDNF+Q), F3A-NT3 (NT-3+Q), or F3A-NT4/5 (NT-4/5+Q) cell lines differentially reduced the volume of the lesion for (A) GAD-, (B) PPE-, and (C) PPTA- but not for (D) DYN-positive neurons. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with MT+Q-injected striata by one-way ANOVA followed by Scheffé post hoc test.

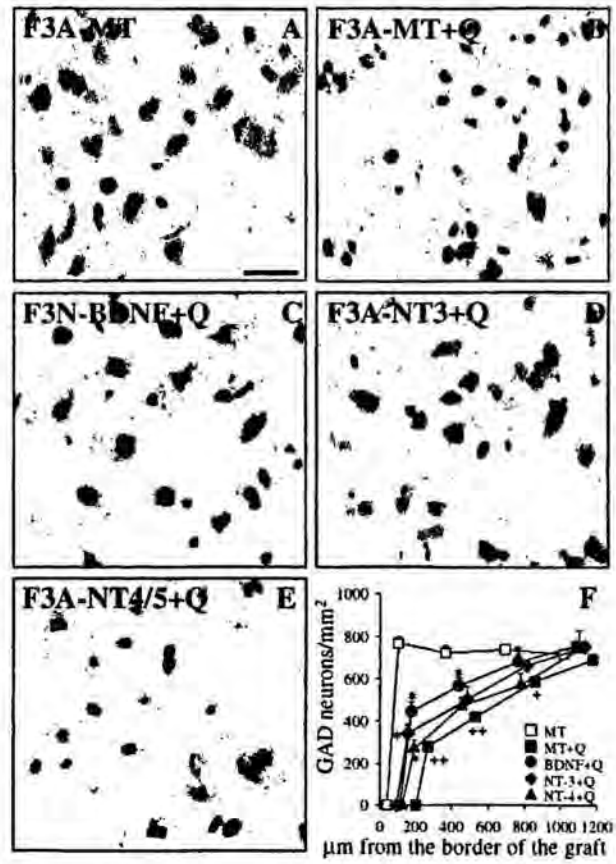

FIG. 5. BDNF was the most efficient neurotrophin at preventing the degeneration of GAD-positive neurons in the QUIN (Q)-lesioned striata. All photomicrographs show GAD-positive neurons in the first region analyzed, next to the graft and lesion sites. **A:** F3A-MT (MT) control. Bar = 20 µm. **B:** Grafting of the MT cell line in Q-injected striata did not prevent loss of GAD-positive neurons. In contrast, grafting of the F3N-BDNF (BDNF) cell line (**C**) and, to a lesser extent, the F3A-NT3 (NT-3; **D**) or F3A-NT4/5 (NT-4; **E**) cell line, prevented the loss of neurons expressing GAD mRNA. **F:** Mean ± SEM (bars) cell density (n = 4 animals per condition). $^{*}p < 0.01$ compared with MT; $^{+}p < 0.05$, $^{++}p < 0.01$ compared with MT+Q by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test.

of neurons and resulted in a specific pattern of surviving striatal projection neurons. Moreover, this pattern was not modified by F3A-MT grafting (Table 1), indicating that the control cell line had no survival-promoting effect per se.

GAD-expressing cells are mainly protected by BDNF

QUIN injection in animals grafted with the control cell line induced a 56, 44, and 22% loss of GAD-positive neurons in regions 1–3, respectively (Fig. 5F). In agreement with the decrease in the lesion size by neurotrophins, intrastriatal grafting of neurotrophin-secreting cell lines prevented, with different efficiency, the decrease in the number of GAD-positive neurons induced by QUIN. Whereas intrastriatal grafting of F3N-BDNF or F3A-NT3 prevented GAD-positive cell loss in all the regions examined, F3A-NT4/5 only protected those neurons in the first region (Fig. 5). The total effect of neurotrophins

TABLE 1. *Grafting of the control cell line did not modify the loss of striatal projection neurons induced by QUIN*

	PPE		GAD		PPTA		DYN	
	QUIN	MT + QUIN	QUIN	MT + QUIN	QUIN	MT + QUIN	QUIN	MT + QUIN
Region 1	127 ± 5	148 ± 23	250 ± 22	279 ± 4	150 ± 22	145 ± 10	188 ± 13	206 ± 29
Region 2	221 ± 15	258 ± 32	350 ± 22	349 ± 13	330 ± 20	314 ± 26	260 ± 29	257 ± 14
Region 3	389 ± 6	399 ± 10	458 ± 20	462 ± 17	417 ± 17	397 ± 27	333 ± 17	352 ± 19
Region 4	522 ± 36	549 ± 10	542 ± 20	551 ± 13	517 ± 17	503 ± 24	467 ± 21	458 ± 14

Data are mean ± SEM cell density (in cells/mm²; n = 4 animals per condition) of identified neurons found in every condition. Region 1 was defined as the first area where positive neurons were found, with other regions progressively scored at 325-µm increments (see Materials and Methods). QUIN, QUIN injection alone; MT + QUIN, F3A-MT + QUIN-injected striatum. Results were statistically analyzed by Kruskal–Wallis ANOVA.

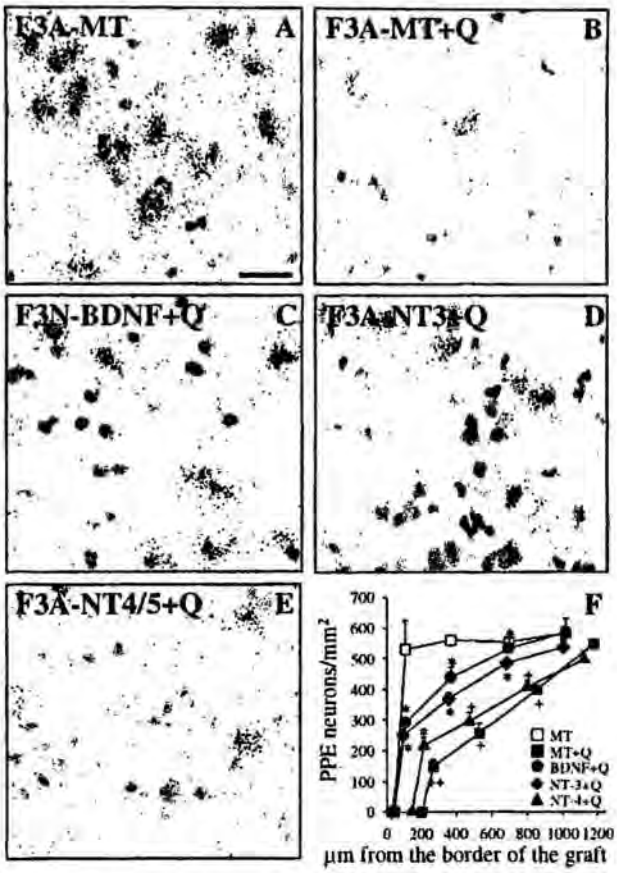

FIG. 6. BDNF, NT-3, and to a lesser extent NT-4/5 prevent the degeneration of PPE-positive neurons in the QUIN (Q)-lesioned striata. All photomicrographs show the first region next to the lesioned area, where the first labeled neurons were found. **A:** F3A-MT (MT) control. Bar = 20 μm. **B:** Grafting of the MT cell line did not prevent PPE-neuron loss induced by Q. Grafting of the F3N-BDNF (BDNF; **C**), F3A-NT3 (NT-3; **D**), or F3A-NT4/5 (NT-4; **E**) cell line differentially prevented the Q-induced degeneration of neurons expressing PPE mRNA. **F:** Mean ± SEM (bars) cell density (n = 4 animals per condition) of PPE-positive neurons. $^{*}p < 0.01$ compared with MT; $^{+}p < 0.05$, $^{++}p < 0.01$ compared with MT+Q by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test.

was also evaluated in the first millimeter from the graft, the area where cell loss takes place. BDNF was the most efficient factor and protected 68% of GAD-positive neurons affected by QUIN. In contrast, NT-3 and NT-4/5 protected GAD-positive neurons by only 34 and 20%, respectively.

PPE-expressing cells are differentially protected by neurotrophins

PPE mRNA-positive neurons were the most severely affected by QUIN-induced excitotoxicity. A 72% cell loss was observed in the first field examined (region 1; compare Fig. 6A with B and see Fig. 6F). Cell loss in regions 2 and 3 was ~54 and 30%, respectively (Fig. 6F). As observed for GAD-positive neurons, implantation of BDNF- or NT-3-secreting cells significantly reduced the loss of PPE-positive neurons in all the regions examined, whereas NT-4/5 only protected these neurons in region 1 (Fig. 6F). In the first millimeter, F3A-BDNF or F3A-NT3 grafting prevented the loss of PPE-positive neurons by 70 and 64%, respectively. In contrast, NT-4/5 only prevented the loss of 27% of PPE-positive neurons.

PPTA-positive neurons are protected by all neurotrophins to a similar extent

PPTA-positive neurons were the most resistant to QUIN. A significant loss of neurons was found only in regions 1 (65%) and 2 (27%) (Fig. 7F). Grafting of F3N-BDNF, F3A-NT3, or F3A-NT4/5 cells prevented the loss of PPTA-positive neurons by 30, 44, or 37%, respectively, in the first millimeter.

DYN-expressing cells are only protected by BDNF

DYN-positive neurons were also lost after QUIN injection. There was ~50, 40, and 20% of reduction in the

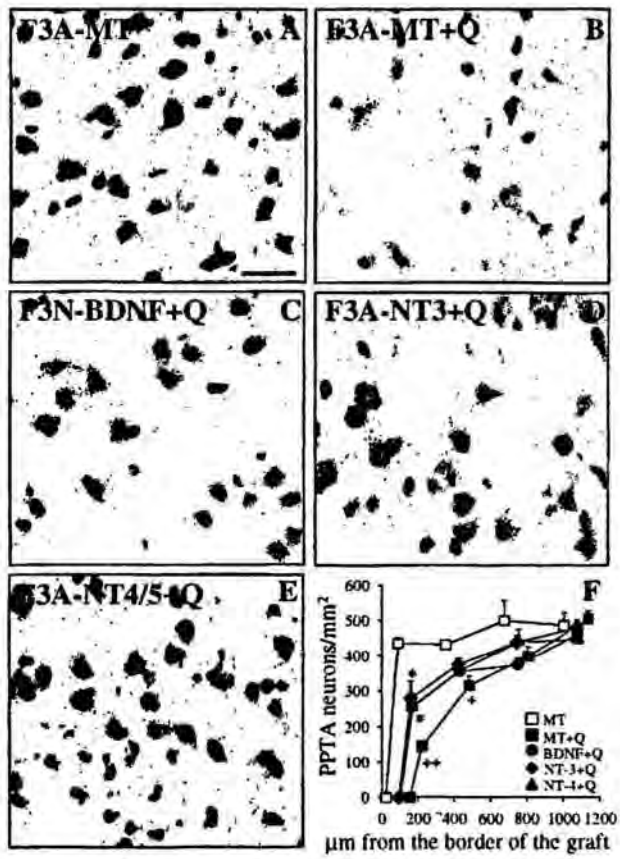

FIG. 7. BDNF, NT-3, and NT-4/5 prevent with similar efficiency the loss of PPTA-positive neurons induced by QUIN (Q) injections. **A:** F3A-MT (MT) control. Bar = 20 μm. **B:** Grafting of the MT cell line did not prevent the loss of PPTA-positive neurons in the Q-injected striata. However, all of the neurotrophin cell lines—F3N-BDNF (BDNF; **C**), F3A-NT3 (NT-3; **D**), and F3A-NT4/5 (NT-4; **E**)—prevented the degeneration of neurons expressing PPTA mRNA. Photomicrographs show the first region where labeled neurons were counted. **F:** Mean ± SEM (bars) density (n = 4 animals per condition) of PPTA-positive neurons. $^{*}p < 0.01$ compared with MT; $^{+}p < 0.05$, $^{++}p < 0.01$ compared with MT+Q by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test.

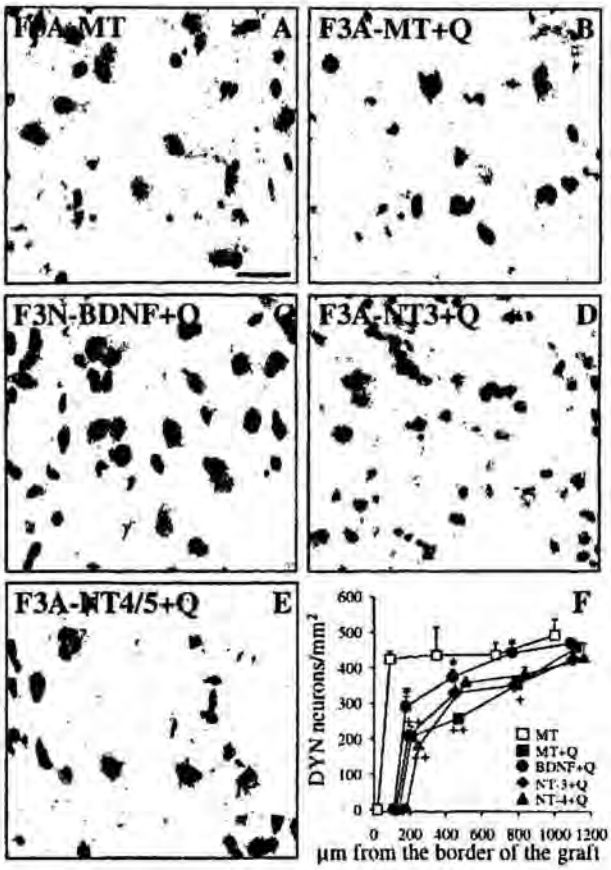

FIG. 8. BDNF prevents the loss of DYN-positive neurons induced by QUIN (Q) excitoxicity. All photomicrographs show the first region where cell counting was performed. **A:** F3A-MT (MT) control. Bar = 20 μm. Grafting of MT (**B**), F3A-NT3 (NT-3; **D**), or F3A-NT4/5 (NT-4; **E**) cell lines did not prevent the loss of DYN-positive neurons in the Q-injected striata. Instead, grafting of the F3N-BDNF cell line (BDNF; **C**) prevented the loss of neurons expressing DYN mRNA. **F:** Mean ± SEM (bars) density (n = 4 animals per condition) of DYN-positive neurons. $^{*}p < 0.01$ compared with MT; $^{+}p < 0.05$, $^{++}p < 0.01$ compared with MT+Q by Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test.

number of DYN-positive neurons in regions 1–3, respectively (Fig. 8F). Only F3N-BDNF grafts prevented the QUIN-induced death of DYN-positive neurons (compare Fig. 8C with B, D, and E, and see Fig. 8F). The overall response in the first millimeter was a protection of ~52% for BDNF.

DISCUSSION

In the present report we show that neurotrophins decrease the number of TUNEL-positive nuclei in the striatum, reduce the lesion size, and differentially prevent the loss of striatal projection neurons from intrastriatal QUIN injection. BDNF is the most efficient factor at preventing cell death and the factor with the broadest neuroprotective activity on striatal projection neurons. In situ hybridization studies show that BDNF can protect all populations of striatal projection neurons from QUIN excitotoxicity. In contrast, NT-3 and NT-4/5 do not prevent the loss of DYN-positive neurons and are less efficient than BDNF at protecting GAD-positive neurons.

Although an indirect action of neurotrophins cannot be ruled out, our results could be explained by a direct action of BDNF, NT-3, and NT-4/5 on striatal projection neurons because they express both TrkB and TrkC but not TrkA receptors (Merlio et al., 1992). In agreement with our data, in vitro studies have shown that BDNF, NT-3, and NT-4/5 promote the survival of striatal GABAergic neurons (Widmer and Hefti, 1994; Ventimiglia et al., 1995), that NT-4/5 prevents the death of substance P-containing striatal neurons (Ardelt et al., 1994), and that neurotrophins protect striatal projection neurons from different types of injury (Nakao et al., 1995*a,b*). With regard to in vivo studies, BDNF (Martínez-Serrano and Björklund, 1996; Bemelmans et al., 1999) and NT-4/5 (Alexi et al., 1997) have been found to prevent the degeneration of striatal projection neurons induced by intrastriatal QUIN injection. However, some other studies failed to detect any effect of BDNF (Frim et al., 1993; Anderson et al., 1996) or NT-3 (Anderson et al., 1996). In the latter cases, methodological differences, including the parameters used to assess neuronal rescue, the severity of the lesion, and/or the high doses of neurotrophins, can account for the absence of effects. In fact, it has been shown that long exposure to high doses of BDNF induces a desensitization of TrkB in vivo (Frank et al., 1996; Knüsel et al., 1996) and down-regulates TrkB mRNA or desensitizes TrkB in vitro (Carter et al., 1995; Frank et al., 1996), suggesting that treatment with high doses of BDNF or NT-3 for extended periods may reduce TrkB and TrkC responsiveness to neurotrophins. Instead, exposure to lower doses of BDNF in vivo does not down-regulate the BDNF response (Knüsel et al., 1996), TrkB expression is induced (Ferrer et al., 1998), and striatal projection neurons are protected from QUIN excitotoxicity (Martínez-Serrano and Björklund, 1996; Bemelmans et al., 1999; present study). Thus, low doses of BDNF seem to be not only sufficient, but also necessary, to activate efficiently TrkB and exploit all the therapeutic potential of this trophic factor.

From a functional point of view, BDNF has been reported to be anterogradely transported to the striatum from the cerebral cortex (Altar et al., 1997), and low levels of neurotrophins are known to be expressed in the striatum and in the targets of striatal projection neurons, pallidum, and substantia nigra (Miranda et al., 1993; Seroogy and Gall, 1993; Timmusk et al., 1993). To explore whether neurotrophins could elicit functional responses on striatal projection neurons from these sites, we have previously delivered neurotrophins either in the substantia nigra (Arenas et al., 1996) or in the striatum (Pérez-Navarro et al., 1999*a*). Our results showed that neurotrophins are able to regulate the phenotype of striatal projection neurons both in a target-derived or in an autocrine/paracrine fashion. In the present study we now

show that neurotrophins can also act within the striatum as autocrine/paracrine survival factors to prevent the degeneration of striatal projection neurons. Such a mode of action is in agreement with the presence of recurrent axon collaterals of striatal projection neurons within the striatum. Comparison of the effects of neurotrophins on the phenotype (Pérez-Navarro et al., 1999*a*) and survival (present study) of striatal projection neurons shows that these functions are differentially regulated by a given neurotrophin, suggesting independent mechanisms of action. For instance, NT-4/5 protected PPE- and GAD-positive neurons only in the first region examined (present study), whereas this neurotrophin also prevented the decrease in the soma area of PPE- and GAD-positive neurons induced by QUIN in all the regions examined (Pérez-Navarro et al., 1999*a*). Furthermore, NT-3 showed greater effects than BDNF and NT-4 at regulating the phenotype of striatal neurons (Pérez-Navarro et al., 1999*a*), whereas we show here that BDNF is the most efficient factor at preventing the loss of striatal neurons. Thus, our results are in agreement with the concept that binding of neurotrophins to their receptors activates different intracellular pathways to induce survival and/or differentiation (for review, see Segal and Greenberg, 1996; Springer and Kitzman, 1998).

Excitotoxicity, oxidative stress, impaired energy metabolism, and caspase activation have been proposed as mechanisms to explain neurodegeneration in HD (Goldberg et al., 1996; Petersén et al., 1999). The last possibility has recently received much attention because inhibition of caspase-1 was found to slow disease progression in a mouse model of HD (Ona et al., 1999). Furthermore, some reports have shown that neurotrophic factors can exert their protective effects by modifying caspase activity in vitro (Tamatani et al., 1998) and in vivo (Han et al., 2000). Thus, all current available data suggest that inappropriate apoptosis may take place in HD and that neurotrophins may be able to prevent cell death by activating similar molecular mechanisms as during development. In agreement with this possibility, the regulation and function of neurotrophin receptors during development and regeneration also seem to be similar. For instance, TrkB protein is developmentally regulated in the striatum (Constantini et al., 1999), and *trk*B-null mutant mice showed increased striatal apoptotic cell death during development (Alcántara et al., 1998). Similarly, we recently found that TrkB expression was up-regulated by intrastriatal QUIN injection (Canals et al., 1999) and that both TrkB ligands, BDNF and NT-4/5, regulate the phenotype of striatal projection neurons (Pérez-Navarro et al., 1999*a*) and promote their survival (present study). Thus, combined, all these data suggest an important role of neurotrophins, and in particular of TrkB ligands, both during development and regeneration of striatal neurons.

In conclusion, the present work shows that BDNF, NT-3, and NT-4/5 differentially prevent the death of identified striatal projection neurons and that BDNF is the most efficient neurotrophin in promoting survival of striatal projection neurons. Thus, our findings suggest that continuous supply of low doses of BDNF might be beneficial for the treatment of neurological disorders affecting striatal projection neurons.

Acknowledgment: We are very grateful to Lotta Johanson for secretarial help, Annika Ahlsen and Maite Muñoz for technical assistance, and Anna Bosch for her support and advice in the use of confocal microscopy. Financial support was obtained from the BIOMED2 Program of the European Commission (grant BMH4-CT96-0273), CICYT (grants SAF96-0043 and SAF99-0019, Ministerio de Educación y Ciencia, Spain), La Marató de TV3 (grant 97-TV1009), the Swedish Medical Research Council (MFR, grant K98-12X-11563-03A), the Karolinska Institute, and the Jeanssonska and the Kapten Arthur Eriksson Foundations. E.P.-N. was a fellow of the European Molecular Biology Organization.

REFERENCES

Alcántara S., Frisén J., del Rio J. A., Soriano E., Barbacid M., and Silos-Santiago I. (1997) TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death. *J. Neurosci.* **17,** 3623–3633.

Alexi T., Venero J. L., and Hefti F. (1997) Protective effects of neurotrophin-4/5 and transforming growth factor-α on striatal neuronal phenotypic degeneration after excitotoxic lesioning with quinolinic acid. *Neuroscience* **78,** 73–86.

Altar C. A., Cai N., Bliven T., Juhasz M., Conner J. M., Acheson A. L., Lindsay R., and Wiegand S. J. (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389,** 856–860.

Anderson K. D., Panayotatos N., Corcoran T. L., and Lindsay R. M. (1996) Ciliary neurotrophic factor protects striatal output neurons in an animal model of Huntington disease. *Proc. Natl. Acad. Sci. USA* **93,** 7346–7351.

Ardelt A. A., Flaris N. A., and Roth K. A. (1994) Neurotrophin-4 selectively promotes survival of striatal neurons in organotypic culture. *Brain Res.* **647,** 340–344.

Arenas E. and Persson H. (1994) Neurotrophin-3 prevents the death of adult central noradrenergic neurons *in vivo*. *Nature* **367,** 368–371.

Arenas E., Åkerud P., Wong V., Boylan C., Persson H., Lindsay R. M., and Altar C. A. (1996) Effects of BDNF and NT-4/5 on striatonigral neuropeptides or nigral GABA neurons *in vivo*. *Eur. J. Neurosci.* **8,** 1707–1717.

Barbacid M. (1994) The Trk family of neurotrophin receptors. *J. Neurobiol.* **25,** 1386–1403.

Beal M. F., Kowall N. W., Ellison D. W., Mazurek M. F., Swartz K. J., and Martin J. B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature* **321,** 168–171.

Bemelmans A. P., Horellou P., Pradier L., Brunet I., Colin P., and Mallet J. (1999) Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, as demonstrated by adenoviral gene transfer. *Hum. Gene Ther.* **10,** 2987–2997.

Canals J. M., Checa N., Marco S., Michels A., Pérez-Navarro E., and Alberch J. (1999) The neurotrophin receptors TrkA, TrkB and TrkC are differentially regulated after excitotoxic lesion in rat striatum. *Mol. Brain Res.* **69,** 242–248.

Carlock L., Walker P. D., Shan Y., and Gutridge K. (1995) Transcription of the Huntington disease gene during the quinolinic acid cascade. *Neuroreport* **6,** 1121–1124.

Carter B. D. and Lewin G. R. (1997) Neurotrophins live or let die: does p75NTR decide? *Neuron* **18,** 187–190.

Carter B. D., Zirrgiebel U., and Barde Y.-A. (1995) Differential regulation of $p21^{ras}$ activation in neurons by nerve growth factor and brain-derived neurotrophic factor. *J. Biol. Chem.* **270,** 21751–21757.

Coggeshall R. E. (1992) A consideration of neuronal counting methods. *Trends Neurosci.* **15,** 9–13.

Connor B. and Dragunow M. (1998) The role of neuronal growth factors in neurodegenerative disorders of the human brain. *Brain Res. Rev.* **27,** 1–39.

Constantini L. C., Feinstein S. C., Radeke M. J., and Snyder-Keller A. (1999) Compartmental expression of trkB receptor protein in the developing striatum. *Neuroscience* **89,** 505–513.

DiFiglia M. (1990) Excitotoxic injury of the neostriatum: a model for Huntington's disease. *Trends Neurosci.* **13,** 286–289.

Ernfors P., Merlio J.-P., and Persson H. (1992) Cells expressing mRNA for neurotrophins and their receptors during embryonic rat development. *Eur. J. Neurosci.* **4,** 1140–1158.

Ferrer I., Martín F., Serrano T., Reiriz J., Pérez-Navarro E., Alberch J., Macaya A., and Planas A. (1995) Both apoptosis and necrosis occur following intrastriatal administration of excitotoxins. *Acta Neuropathol. (Berl.)* **90,** 504–510.

Ferrer I., Ballabriga J., Marti E., Pérez-Navarro E., Alberch J., and Arenas E. (1998) BDNF up-regulates trkB protein and prevents the death of CA1 neurons following transient forebrain ischemia. *Brain Pathol.* **8,** 253–261.

Frank L., Ventimiglia R., Anderson K., Lindsay R. M., and Rudge J. S. (1996) BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. *Eur. J. Neurosci.* **8,** 1220–1230.

Frim D. M., Uhler T. A., Short M. P., Ezzedine Z. D., Klagsbrun M., Breakefield X. O., and Isacson O. (1993) Effects of biologically delivered NGF, BDNF and bFGF on striatal excitotoxic lesions. *Neuroreport* **4,** 367–370.

Goldberg Y. P., Nicholson D. W., Rasper D. M., Kalchman M. A., Koide N. A., Vaillancourt J. P., and Hayden M. R. (1996) Cleavage of huntingtin by apopain, a preapoptotic cystein protease, is modulated by the polyglutamate tract. *Nat. Genet.* **13,** 442–449.

Han B. H., D'Costa A., Back S. A., Parsadanian M., Patel S., Shah A. R., Gidday J. M., Srinivasan A., Deshmukh M., and Holtzman D. M. (2000) BDNF blocks caspase-3 activation in neonatal hypoxia–ischemia. *Neurobiol. Dis.* **7,** 38–53.

Hefti F. (1994) Neurotrophic factor therapy for nervous system degenerative disorders. *J. Neurobiol.* **25,** 1418–1435.

Hughes P. E., Alexi T., Yoshida T., Schreiber S. S., and Knusel B. (1996) Excitotoxic lesion of rat brain with quinolinic acid induces expression of p53 messenger RNA and protein and p53-inducible genes *BAX* and *GADD-45* in brain areas showing DNA fragmentation. *Neuroscience* **74,** 1143–1160.

Knüsel B., Kaplan D. R., and Hefti F. (1996) Intraparenchymal NGF injections in adult and aged rats induce long-lasting Trk tyrosine phosphorylation. *Exp. Neurol.* **139,** 121–130.

MacDonald M. E. and Gusella J. F. (1996) Huntington's disease: translating CAG repeat into a pathogenic mechanism. *Curr. Opin. Neurobiol.* **6,** 638–643.

Mangiarini L., Sathasivam K., Seller M., Cozens B., Harper A., Hetherington C., Lawton M., Trottier Y., Lehrach H., Davies S. W., and Bates G. P. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neuropathological phenotype in transgenic mice. *Cell* **87,** 493–506.

Martínez-Serrano A. and Björklund A. (1996) Protection of the neostriatum against excitotoxic damage by neurotrophin-producing, genetically modified neural stem cells. *J. Neurosci.* **16,** 4604–4616.

Merlio J.-P., Ernfors P., Jaber M., and Persson H. (1992) Molecular cloning of rat *trk*C and distribution of cells expressing messenger RNAs for members of the *trk* family in the rat central nervous system. *Neuroscience* **51,** 513–532.

Miranda R. C., Sohrabji F., and Toran-Allerand D. (1993) Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. *Proc. Natl. Acad. Sci. USA* **90,** 6439–6443.

Mufson E. J., Kroin J. S., Sendera T. J., and Sobreviela T. (1999) Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases. *Prog. Neurobiol.* **157,** 451–484.

Nakao N., Kokaia Z., Odin P., and Lindvall O. (1995*a*) Protective effects of BDNF and NT-3 but not PDGF against hypoglycemic injury to cultured striatal neurons. *Exp. Neurol.* **131,** 1–10.

Nakao N., Brundin P., Funa K., Lindvall O., and Odin P. (1995*b*) Trophic and protective actions of brain-derived neurotrophic factor on striatal DARPP-32 containing neurons in vitro. *Dev. Brain Res.* **90,** 92–101.

Neveu I. and Arenas E. (1996) Neurotrophins promote the survival and development of neurons in the cerebellum of hypothyroid rats in vivo. *J. Cell Biol.* **133,** 631–646.

Ona V. O., Li M., Vonsattel J. P. G., Andrews L. J., Khan S. Q., Chung W. M., Frey A. S., Menon A. S., Li X.-J., Stieg P. E., Yuan J., Penney J. B., Young A. B., Cha J.-H. J., and Friedlander R. M. (1999) Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* **399,** 263–267.

Pérez-Navarro E., Arenas E., Reiriz J., Calvo N., and Alberch J. (1996) Glial cell-line derived neurotrophic factor (GDNF) protects striatal calbindin immunoreactive neurons from excitotoxic damage. *Neuroscience* **75,** 345–352.

Pérez-Navarro E., Alberch J., Neveu I., and Arenas E. (1999*a*) BDNF, NT-3 and NT-4/5 differentially regulate the phenotype and prevent degenerative changes of striatal projection neurons after excitotoxicity *in vivo*. *Neuroscience* **91,** 1257–1264.

Pérez-Navarro E., Arenas E., Marco S., and Alberch J. (1999*b*) Intrastriatal grafting of a GDNF-producing cell line protects striatonigral neurons from quinolinic acid excitotoxicity *in vivo*. *Eur. J. Neurosci.* **11,** 241–249.

Petersén Å., Mani K., and Brundin P. (1999) Recent advances in the pathogenesis of Huntington's disease. *Exp. Neurol.* **157,** 1–18.

Portera-Cailliau C., Hedreen J. C., Price D. L., and Koliatsos V. E. (1995) Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. *J. Neurosci.* **15,** 3775–3787.

Reiner A., Albin R. L., Anderson K. D., D'Amato C. J., Penney J. B., and Young A. B. (1988) Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl. Acad. Sci. USA* **85,** 5733–5737.

Richfield E. K., Maguire-Zeiss K. A., Vonkeman H. E., and Voorn P. (1995) Preferential loss of preproenkephalin versus preprotachykinin neurons from the striatum of Huntington's disease patients. *Ann. Neurol.* **38,** 852–861.

Segal R. A. and Greenberg M. E. (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu. Rev. Neurosci.* **19,** 463–489.

Segal R. A., Bhattacharyya A., Rua L. A., Alberta J. A., Stephens R. M., Kaplan D. R., and Stiles C. D. (1996) Differential utilization of Trk autophosphorylation sites. *J. Biol. Chem.* **271,** 20175–20181.

Seroogy K. B. and Gall C. (1993) Expression of neurotrophins by midbrain dopaminergic neurons. *Exp. Neurol.* **124,** 119–128.

Springer J. E. and Kitzman P. H. (1998) Neuroprotective strategies involving the neurotrophins and their signaling pathways, in *Neuroprotective Signal Transduction* (Mattson M. P., ed), pp. 1–22. Humana Press, Totowa, New Jersey.

Tamatani M., Ogawa S., and Tohyama M. (1998) Roles of Bcl-2 and caspases in hypoxia-induced neuronal cell death: a possible neuroprotective mechanism of peptide growth factors. *Mol. Brain Res.* **58,** 27–39.

Tatter S. B., Galpern W. R., Hoogeveen A. T., and Isacson O. (1995) Effects of striatal excitotoxicity on huntingtin-like immunoreactivity. *Neuroreport* **6,** 1125–1129.

Timmusk T., Belluardo N., Metsis M., and Persson H. (1993) Widespread and developmentally regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. *Eur. J. Neurosci.* **5,** 605–613.

Thomas L. B., Gates D. J., Richfield E. K., O'Brien T. F., Schweitzer J. B., and Steindler D. A. (1995) DNA end labeling (TUNEL) in Huntington's disease and other neuropathological conditions. *Exp. Neurol.* **133,** 265–272.

Ventimiglia R., Mather P. E., Jones B. E., and Lindsay R. M. (1995) The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons *in vitro*. *Eur. J. Neurosci.* **7,** 213–222.

Widmer H. R. and Hefti F. (1994) Neurotrophin-4/5 promotes survival and differentiation of rat striatal neurons developing in culture. *Eur. J. Neurosci.* **6,** 1669–1679.

AUTOCRINE/PARACRINE TROPHIC RESPONSE IN CORTICAL NEURONS IS INDUCED BY STRIATAL INJURY

Article sotmès a revisió

Autocrine/Paracrine Trophic Response in Cortical Neurons Is Induced by Striatal Injury

Josep M. Canals[1], Anna M. Canudas[1,2], Núria Checa[1], Mercè Pallàs[2] and Jordi Alberch[1]

[1]Departament de Biologia Cel·lular i Anatomia Patològica, Facultat de Medicina, Universitat de Barcelona, IDIBAPS, Casanova 143, E-08036 Barcelona, Spain. [2]Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari Pedralbes, Barcelona, Spain

Correspondence should be addressed to Dr. Jordi Alberch, Departament de Biologia Cel·lular i Anatomia Patològica, Facultat de Medicina, Universitat de Barcelona, Casanova 143, E-08036 Barcelona, Spain. Tel: 34-934035285; Fax: 34-934021907; e-mail: alberch@medicina.ub.es

Running title: Autocrine/paracrine cortical trophic regulation

The cerebral cortex expresses multiple members of the neurotrophin family and their receptors, which participate in the establishment and maintenance of neuronal circuits. Here we describe cortical changes in the expression of brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and their receptors after intrastriatal injection of several glutamate receptor agonists: quinolinate, kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and trans-(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD). Each neurotrophin and receptor showed specific patterns of expression in cortical layers depending on the agonist injected. Changes in BDNF and NT-3 mRNA levels were induced in cortical neurons only when the striatum was lesioned with quinolinate or kainate. However, whereas kainate increased the expression of both neurotrophins, the striatal injection of quinolinate upregulated BDNF but downregulated NT-3. In contrast, both TrkB isoforms increased in the cortex after intrastriatal injection of each glutamate receptor agonist. Changes in cortical TrkB expression were ligand-independent and were not related to the severity of striatal damage. Increased levels of TrkC were observed after intrastriatal injection of those glutamate receptor agonists, AMPA or ACPD, which did not modify the levels of cortical NT-3 expression. In conclusion, the present findings show a complex trophic response in the cortex after striatal injury, suggesting that cortical neurons use multiple and specific neurotrophic signals to direct adult plasticity.

Introduction

Each layer of mammalian cerebral cortex contains a unique assembly of neuronal types with characteristic axonal projections, physiological properties, and dendritic arbors. Development of these neuronal populations is specifically regulated by members of the neurotrophin family which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), and dendritic growth response to each neurotrophin differs across the six layers of the cortex (Snider 1994; Bonhoeffer 1996; Reichardt and Fariñas, 1997; McAllister *et al.*, 1999). Cortical layers display different patterns of expression of neurotrophins and their receptors during development and in adulthood (Merlio *et al.*, 1992; Miranda *et al.*, 1993; Schmidt-Kastner *et al.*, 1996; Friedman *et al.*, 1998; Pitts and Miller, 2000). The regulation of neurotrophins and their receptors, TrkA, TrkB and TrkC, contributes to the maintenance of the adult cortex (Yuste and Sur, 1999; Xu *et al.*, 2000). Moreover, BDNF and NT-3 have been implicated in the regulation of adult synaptic activity (Thoenen 1995; Bonhoeffer 1996; McAllister *et al.*, 1999; Lodovichi *et al.*, 2000). Thus, BDNF increases the stimulation of evoked release of glutamate (Takei *et al.*, 1997), whereas NT-3 reduces the synaptic transmission mediated by γ-aminobutyric acid (GABA) in cortical neurons (Kim *et al.*, 1994). It has been postulated that reciprocal regulation between neurotrophin expression and neuronal activity may operate to modulate synaptic efficacy (Kim *et al.*, 1994).

Several lines of evidence implicate neurotrophins as mediators of cortical structural plasticity in degenerative paradigms (Lindvall *et al.*, 1994; Giehl *et al.*, 1998; Kokaia *et al.*, 1998; Schütte *et al.*, 2000; Canals *et al.*, 2001). It has also been shown that levels of BDNF mRNA increase in cortical neurons after brain insults, including epileptic seizures, cerebral ischemia, excitotoxicity and traumatic injury (Lindvall *et al.*, 1994; Isackson 1995; Rocamora *et al.*, 1996; Kokaia *et al.*, 1998; Checa *et al.*, 2000; Canals *et al.*, 2001). In contrast, seizure activity reduces NT-3 mRNA expression in cortical and hippocampal neurons (Castren *et al.*, 1993; Lindvall *et al.*, 1994; Rocamora *et al.*, 1994; Elmer *et al.*, 1996). Transient changes in the high affinity neurotrophin receptors, Trks, have also been described after similar manipulations (Bengzon *et al.*, 1993; Merlio *et al.*, 1993). As neurotrophins are reported to protect against cortical damage (Cheng and Mattson, 1994; Kume *et al.*, 1997), endogenous changes in neurotrophin expression may be a neuroprotective response (Lindvall *et al.*, 1994). Furthermore, it has been postulated that enhancement of BDNF in the cortex may be involved in protection of striatal neurons against

damage via anterograde transport through the corticostriatal pathway (Altar and DiStefano, 1998; Kokaia *et al.*, 1998; Canals *et al.*, 2001).

The corticostriatal connection is formed by afferents from all areas of the cerebral cortex, which regulate neuronal activity in striatal neurons via glutamate release. Pyramidal neurons in layer V provide the main neo- and mesocortical projection to the striatum, but additional inputs come from layers II/III and VI (Gerfen 1992). Glutamate, the most prevalent excitatory amino acid in the brain, activates both ionotropic and metabotropic receptor subtypes. Excessive activation of glutamate receptors in the striatum produces selective degeneration of striatal projection neurons which resembles Huntington's disease (HD) (DiFiglia 1990; Beal *et al.*, 1991). The lesions are confined to neuronal cell bodies, do not affect traversing or afferent fibers and appear to spare non-neuronal elements such as glia (Beal *et al.*, 1991). However, this neurodegenerative disease does not only affect the striatum. In HD cerebral cortex a reduction of area (de la Monte *et al.*, 1988) and a loss of pyramidal neurons (Cudkowicz and Kowall, 1990) have been described. Whether this degeneration is a primary effect of the illness or secondary to striatal degeneration is unknown.

Recent reports suggest that the pathogenesis of some human neurodegenerative disorders is due to an alteration in neurotrophic factor or Trk receptor levels (for review: Connor and Dragunow, 1998). Reduction in BDNF has been described in the brains of HD patients (Ferrer *et al.*, 2000). Moreover, the levels of BDNF, NT-3 and their receptors are also reduced in post-mortem cortex and hipocampus of Alzheimer's disease patients (Connor and Dragunow, 1998). These findings indicate that strategies that increase the levels of neurotrophic factors and their receptors could enhance neuronal survival during neurodegenerative processes. Here we examine endogenous changes in the expression of BDNF, NT-3 and their receptors in the cerebral cortex after intrastriatal injection of several glutamate receptor agonists. We observed specific patterns of regulation of expression for neurotrophins and their receptors in the cortex ipsilateral to each agonist-injected striatum. Since the doses we used do not produce cortical degeneration (Canals *et al.*, 2001), our findings indicate that a combined trophic response may be involved in the protection of cortical neurons after target striatal damage.

Materials and Methods

Animal treatment.

Male Sprague-Dawley rats (170-210 g) were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a David Kopf stereotaxic apparatus (DK 900) with the incisor bar 5 mm above the interaural line. We used 4-7 animals for each condition and time point studied. Glutamate receptor agonists: quinolinate (QUIN, 68 nmol, Sigma Chemicals Co., St. Louis, MO), kainate (KA, 6 nmol, Sigma), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA, 6 nmol, Research Biochemicals International (RBI), Natick, MA) or trans-(1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 100 nmol, RBI) were injected in a volume of 1 µl into the left striatum at two coordinates (AP (anterioposterior), +2.3 and L (lateral), -2.4 from bregma, and DV (dorsoventral) - 5.0 from dura; AP, +1.3 and L, -3.0 from bregma, and DV - 5.0 from dura) as reported elsewhere (Canals *et al.*, 1998). Agonists were injected at 0.5 µl/min, and the needle was left in place for a further 3 min before being slowly withdrawn. Control animals were injected with 1 µl of phosphate buffered saline (PBS), pH, 7.5.

After lesioning, animals were housed separately with access to food and water *ad libitum* in a colony room maintained at constant temperature (19-22°C) and humidity (40-50%) on a 12:12 h light/dark cycle. All animal-related procedures were in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals and approved by the local animal care committee (C.E.E.A.; Comitè Ètic d'Experimentació Animal) of the Universitat de Barcelona (99/01) and by the Generalitat de Catalunya (99/1094).

RNase protection assay.

Rats were deeply anesthetized in a CO_2 chamber and killed by decapitation at 2, 4, 6, 10, 16 or 24 h after injury. Brains were removed and the cerebral cortex was quickly dissected out, frozen on dry ice, and stored at -80 °C. Total RNA was isolated by the guanidine isothiocyanate method (Chomczynski and Sacchi, 1987). Neurotrophin and Trk mRNA levels were estimated by RNase protection assay using the RPAII Kit (Ambion Inc., Austin, TX) as described previously (Canals *et al.*, 1998; Canals *et al.*, 1999). Antisense cRNA probes for BDNF, NT-3, TrkB and TrkC were prepared as described (Laurenzi *et al.*, 1994; Lindefors *et al.*, 1995). We used a probe (*TrkB all*)

for TrkB that recognizes the full-length and the truncated form of TrkB receptor separately (Lindefors *et al.*, 1995), and the probe used in the present study for TrkC recognizes all isoforms of the receptor. To standardize the amount of mRNA in each lane, an antisense cRNA probe detecting the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcript was prepared as described elsewhere (Canals *et al.*, 1998). In each reaction, total RNA (25 μg for BDNF and NT-3 and 15 μg for TrkB and TrkC) was hybridized at 42°C for 16 h to one of the neurotrophin or neurotrophin receptor antisense cRNA probes together with the GAPDH cRNA probe. Protected fragments were separated on 4% polyacrylamide gels in denaturing conditions, and BIOMAX (Eastman Kodak, Rochester, NY) films were exposed to the gels at -80°C with an intensifying screen for 15 days. The optical density of the autoradiograms was quantified by Phoretix 1D gel analysis (Phoretix International Ltd., Newcastle, UK). Results were normalized to the mean of sham-injected animals and expressed as a percentage of these data. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by LSD (least significant difference *t* test) post-hoc test.

In situ hybridization studies.

In order to localize the changes in the expression of neurotrophins and their receptors, we performed *in situ* hybridization at the time point when mRNA levels were maximal. Thus, animals were killed 4, 6 or 10 h after injection depending on the glutamate receptor agonist injected and on the neurotrophin or receptor analyzed. Brains were dissected out and frozen in dry-ice cooled isopentane. Serial horizontal cryostat sections (14 μm) were processed for hybridization with a radioactive riboprobe as follows. First, tissue sections were postfixed in 4% paraformaldehyde for 15 min. After three washes in PBS, slices were deproteinated in 0.2 M HCl for 10 min, acetylated with 0.25% acetic anhydride in 0.1 M ethanolamine for 10 min and dehydrated with increasing concentrations of ethanol. Sections were incubated for 16 h in a humidified chamber at 53°C with 3 x 10^5 cpm of antisense probe in 150 μl of hybridization cocktail (50% formamide, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0, 0.3 M NaCl, 0.1 M dithiothreitol, 0.5 mg/ml yeast tRNA, 0.1 mg/ml polyA RNA, 1x Denhardt's solution, and 10% dextran sulfate). Antisense cRNA probe to detect the transcripts of BDNF, NT-3, TrkB or TrkC was prepared by *in vitro* transcription using T7 or T3 RNA polymerase (Promega, Madison, WI) and [^{35}S]-UTP (Amersham Pharmacia Biotech, Upsala, Sweden). We used two probes to localize

the changes in expression of TrkB receptor. The first probe was the same that was used in RNase protection assay (*TrkB all*), which recognizes the two isoforms of BDNF receptor and the second one was a specific probe for the tyrosine-kinase domain, which detects only full-length receptor (*TrkB kin*). For TrkC receptor we used a probe that detects all isoforms of this receptor. For control experiments, sense cRNA probe was obtained by *in vitro* transcription. After hybridization, slices were first washed at room temperature in 1x SSC followed by two washes in 1x SSC at 63°C for 15 min each. Single-stranded RNA was digested by RNase treatment (40 μg/ml) for 30 min at 37°C in 0.5 M NaCl, 20 mM Tris-HCl, pH 7.6, 2 mM EDTA. Tissue was washed twice in 1x SSC in 63°C for 10 min and then dehydrated in ethanol and air-dried. β-Max x-ray film (Amersham Pharmacia Biotech) was exposed to slices for 20-30 days. The sections were dipped in LM-1 photoemulsion (Amersham Pharmacia Biotech), exposed at 4°C for 2 months, developed with D19 (Kodak), fixed and counterstained with cresyl violet staining.

Triple labeling for mRNA expression and NeuN and GFAP immunohistochemistry

Some sections were processed for immunohistochemistry after the *in situ* hybridization. Immediately after the last wash in SSC, the slides were coincubated with the primary antibodies to GFAP (1:500, Dako A/S, Glostrup, Germany) and NeuN (1:100, Chemicon, Temecula, CA) overnight at 4°C as described (Canals *et al.*, 2001). After three washes in PBS, the sections were coincubated with both secondary antibodies (anti-rabbit-FICT conjugated, 1:100, Vector laboratories, Burlingame, CA; and anti-mouse-Texas Red conjugated, 1:100, Jackson Immunoresearch Laboratories Inc., West Grove, PE), washed overnight in PBS and dipped as mentioned above. Triple labeling analysis was performed using a confocal microscope.

Results

BDNF and NT-3 are distinctly regulated by cortical neurons after striatal excitotoxicity

To examine BDNF and NT-3 expression in the cerebral cortex following intrastriatal injection of QUIN, KA, AMPA or ACPD, we performed a sensitive RNase protection assay. Intrastriatal injection of QUIN or KA produced different changes in BNDF and NT-3 expression in the cerebral cortex (Fig. 1*A, B*). In contrast, striatal AMPA or ACPD induced no changes in cortical expression of these two neurotrophins (Fig. 1*C, D*). The lesion of the striatum with QUIN

increased BDNF expression to three times the basal levels at 6 h. However, NT-3 expression decreased in the ipsilateral cortex at the same time point (30 ± 3%). Intrastriatal injection of KA upregulated both BDNF and NT-3 cortical mRNA levels, with a maximum at 6 h (524 ± 115%) and 10 h (203 ± 64%), respectively.

In order to localize the cortical changes of neurotrophin expression we performed *in situ* hybridization studies. High levels of BDNF expression were detected in the cortex ipsilateral to QUIN- or KA-lesioned striata (Fig. 2*A*, *B*). In this case, both glutamate receptor agonists produced a similar pattern of expression along the cerebral cortex (Fig. 2*A, B*). Low levels of NT-3 expression were detected by *in situ* hybridization in the contralateral (non-injected) hemisphere (Fig. 2*C, D; right hemisphere*), which hindered the identification of areas where NT-3 was downregulated. We detected decreased levels of NT-3 expression only in the piriform cortex ipsilateral to QUIN-injected striata (Fig. 2*C, E*). In the KA injected animals, NT-3 expression was increased in layer VI of the frontal and parietal cortex, and in layer II-III of the entorhinal cortex. In these areas, we localized the NT-3 upregulation in neurons (NeuN-positive cells) but not in astroglial cells (GFAP-positive cells) (Fig. 2*F, I*). Remarkably, no change of BDNF or NT-3 expression was observed in the hippocampus, a high susceptible zone to the excitotoxicity (Fig. 2*A-D*), which does not connect to the striatum.

Thus, our findings showed a differential regulation of cortical BDNF and NT-3 in response to striatal damage by glutamate receptor agonists. We therefore tested whether the expression of the high-affinity receptors for these neurotrophins was also modified by striatal insult.

Cortical TrkB mRNA upregulation is induced in the cerebral cortex after injection of all glutamate receptor agonists into the striatum

In the RNase protection assay, all four glutamate receptor agonists produced an early and transient increase of expression of both TrkB isoforms with a similar pattern (Fig. 3). Intrastriatal injection of QUIN increased cortical levels of full-length TrkB expression to about twice the control levels at 6 h (Fig.3*A*). The truncated form of TrkB receptor was also upregulated with the maximum 4 h after striatal lesion (Fig. 3*A*). Similarly, animals injected with KA or AMPA showed increased levels of both TrkB isoforms (Fig. 3*B* and *C*, respectively). The greatest increase in the full-length TrkB receptor was observed 4 h after injection of KA (243 ± 57%; Fig. 3*B*). ACPD also increased both forms of TrkB receptor (Fig. 3*D*). The intrastriatal injection

of ACPD showed a high increase of full-length TrkB mRNA, to maximal levels at 2 h (228 ± 38%; Fig. 3*D*). The cortical expression of truncated TrkB receptor also increased between 4 and 6 h after intrastriatal injection of ACPD (194 ± 38 % and 174 ± 41%, respectively; Fig. 3*D*).

In situ hybridization studies showed the same profile of TrkB receptor expression in cortical areas after striatal injection of each glutamate receptor agonist tested (Fig. 4 and 5). However, the two probes used (*TrkB kin* and *TrkB all*) showed different patterns of expression (Fig. 5). Using the *TrkB kin*-probe, we detected that the TrkB isoform that contain the catalytic domain was mainly upregulated in neocortical layers II-III, V and VI (Fig. 5). Full-length TrkB receptor levels were highest in the cortex of KA injected animals, mainly in layer II-III (Fig. 5*C*). However, *TrkB all*-probe revealed TrkB expression in all layers except in the molecular. These results demonstrate that only the truncated form was upregulated in layer IV, since no increase in expression was detected for the *TrkB kin*-probe (Fig. 5*B-E*). However, we cannot elucidate whether the truncated isoform is also expressed in layers II-III, V and VI.

Triple labeling showed that the increase in the high-affinity receptor for BDNF was mainly localized in cortical neurons (Fig. 6). There were no differences at the cellular level between the two probes used for *in situ* hybridization (compare Fig. 6*A* and *B*). High levels of TrkB expression were detected in NeuN-positive cells, whereas no changes in expression were observed in GFAP-positive cells (Fig. 6).

TrkC expression was upregulated in the cortex after intrastriatal injection of AMPA or ACPD

TrkC is the high-affinity neurotrophin receptor preferred by NT-3. We studied whether this receptor was selectively upregulated in the cerebral cortex after intrastriatal injection of glutamate receptor agonists. There was an increase in the expression of TrkC following AMPA or ACPD striatal lesion (Fig. 7*C, D*). Intrastriatal injection of AMPA produced cortical TrkC upregulation between 4 and 6 h (Fig. 7*C*). This increase was 257 ± 50% over control levels at 6 h, when the levels were maximal. The highest levels of cortical TrkC after ACPD striatal lesion were also observed at 6 h, reaching a 215 ± 55% over PBS injected animals (Fig. 7*D*).

High levels of TrkC mRNA were observed in the cingulate cortex ipsilateral to AMPA- or ACPD-injected striata (Fig. 8*C, D*). This upregulation of TrkC expression was localized to cortical neurons of the cingulate area after intrastriatal injection of AMPA (Fig. 8*E*) or ACPD

(data not shown). Levels of TrkC expression were also increased in the layers II-III, V and VI from fronto-parietal to temporal cortex (Fig. 8*F-H*). In these cortical areas, we observed colocalization of TrkC expression on NeuN-positive cells (data not shown). No expression of TrkC was detected in cortical layer IV (Fig. 8*F-H*).

Discussion

Here we demonstrate that striatal damage produced by glutamate receptor agonists stimulates a specific autocrine/paracrine trophic response in the cerebral cortex. This transynaptic regulation differentially affects neurotrophins and their receptors, and it is localized to neurons in particular cortical layers. These results indicate that a complex endogenous trophic response is induced in the cortex following target striatal damage.

The expression of BDNF and NT-3 in cortex was modified after intrastriatal injection of the N-methyl-D-aspartate (NMDA) receptor agonist, QUIN, or the non-NMDA, KA. In contrast, no such change was induced by AMPA or ACPD, which act through non-NMDA and metabotropic glutamate receptors, respectively. Although cortical BDNF and NT-3 mRNA levels were upregulated by striatal KA-injection, these changes were localized to different regions of the cerebral cortex. In contrast, striatal excitotoxicity induced by QUIN upregulated the expression of cortical BDNF but downregulated the levels of NT-3 mRNA in cortex. Differences in the regulation of BDNF and NT-3 mRNA levels have been described previously in other brain nuclei (Rocamora *et al.*, 1994; Canals *et al.*, 1998). Moreover, BDNF and NT-3 have opposite effects on cortical dendritic arbors (McAllister *et al.*, 1997). Thus, these changes in expression of NT-3 and BDNF may reflect the importance of each neurotrophin for specific cortical areas, as they have been described previously in different excitotoxic paradigms (Rocamora *et al.*, 1994; Checa *et al.*, 2000). Taken together, these findings indicate that specific and combined regulation of neurotrophins is induced depending on the neuronal populations affected in the striatum. In fact, trophic dependencies in the adult CNS may be altered by several insults and that the lack of target support could be compensated by the enhancement of autocrine/paracrine trophic factors (Giehl *et al.*, 1998; Schütte *et al.*, 2000).

Trophic support can also be obtained by regulating the expression of neurotrophin receptors. Thus, both the full-length and truncated forms of cortical TrkB receptor were upregulated after

lesioning the striatum. Controversial roles have been attributed to the truncated form in the CNS. Mechanistically, truncated receptors might function as negative regulators of full-length TrkB as demonstrated *in vitro* (Biffo *et al.*, 1995; Eide *et al.*, 1996; Fryer *et al.*, 1997). This function has been attributed to non-neuronal cells (Fryer *et al.*, 1997) that express one of the two truncated isoforms, the T1 (Middlemas *et al.*, 1991; Frisén *et al.*, 1993). Alternatively, truncated TrkB receptors may induce their own signaling independent of the full-length receptor form (Baxter *et al.*, 1997). Moreover, full-length TrkB and the other truncated form, T2 (Middlemas *et al.*, 1991), were mainly detected in neurons (Frisén *et al.*, 1993; Armanini *et al.*, 1995; Biffo *et al.*, 1995; Alderson *et al.*, 2000). Our present results showed that the increase of both forms took place in cortical neurons following striatal injury. In addition, the upregulation of the truncate receptor in layer IV suggest a direct trophic action through this isoform of TrkB. Thus, the different pattern of expression of the two forms of this receptor in cortical layers could be attributed to specific requirements of each neuronal population. Indeed, it has been described that truncated and full-length TrkB receptors regulate distinct modes of dendritic growth (Yacoubian and Lo, 2000).

TrkC, the high-affinity receptor for NT-3 showed a high specific response to the glutamate receptor stimulated, since it only upregulated following striatal injection of AMPA or ACPD. Consistent with these results, TrkB and TrkC receptors show differential regulation in various brain injury paradigms such as kindling- or KA-induced seizures (Barbany and Persson, 1993; Merlio *et al.*, 1993). In addition, the glutamate receptor agonists that induced changes in TrkC mRNA did not change the NT-3 expression and *vice-versa.* These results indicate that cortical neurons may modify their trophic dependencies either by changing the levels of NT-3 or the expression of the high-affinity receptor, TrkC.

The major hypotheses for the functional effects of the insult-induced neurotrophin changes are protection against neuronal damage and stimulation of sprouting and synaptic reorganization (Lindvall *et al.*, 1994). In particular, it has been proposed that changes in cortical BDNF could play a role in survival of cortical and striatal neurons (Altar and DiStefano, 1998; Kokaia *et al.*, 1998; Canals *et al.*, 2001). However, the present findings demonstrating a specific regulation of Trk receptors in the cortex argue in favor of local trophic actions. Therefore, the increase of neurotrophins and Trk receptors might constitute an autocrine/paracrine trophic response of cortical neurons since BDNF and NT-3 protect these cells from various types of insult (Kume *et*

al., 1997). In keeping with this view, no loss of cortical neurons was observed after intrastriatal excitotoxic lesion, suggesting that other sources of trophic factors could supply the lack of the target-area (Canals *et al.*, 2001).

Variations in the expression of neurotrophins and their receptors can also be interpreted as plastic changes and refinement of the efficacy of synaptic transmission (for review: McAllister *et al.*, 1999). Our results show an independent regulation of cortical neurotrophins and their receptors after excitotoxic lesion in the striatum. Moreover, we did not observe any correlation between the severity of the striatal damage and the regulation of Trk receptors in cortex. Thus, another plausible explanation is that activity-dependent regulation of neurotrophic factors modulates a plastic adaptation in the cortex. The injection of glutamate receptor agonist in the striatum modifies a complex network formed by discrete and finely arranged connections through the cortico-basal ganglia-cortical loop (Parent and Hazrati, 1995). This circuitry is essential for the proper selection and execution of a desired movement through a sequence of excitatory and inhibitory events. BDNF and NT-3 seem particularly qualified to mediate activity-dependent interactions between cortical neurons (Bonhoeffer 1996; Marty *et al.*, 1997; McAllister *et al.*, 1999). Therefore, our results indicate that cortical changes in neurotrophin expression and their receptors after striatal injection may participate in the modulation of synaptic transmission in the cortico-basal ganglia-cortical loop.

In conclusion, the present data demonstrate a highly specific cortical trophic response to the lesion of its target-area, the striatum, suggesting that a refinement of trophic signals modulates adult plasticity in the cortex. Analyses of neurotrophin expression in animal models of neurodegenerative diseases may help future understanding of the mechanisms responsible for brain disease and the development of neuroprotective treatments. Stimulation of endogenous production of neurotrophins and Trk receptors may be a good therapeutic approach to prevent the progression of cortical neurodegenerative diseases.

Acknowledgements

We thank M.Teresa Muñoz for technical assistance and Anna Bosch and the "Serveis Científico-Tècnics" of the Universitat de Barcelona for support and advice in the use of confocal

microscopy. We are also very grateful to Robin Rycroft for English language revision. This study was supported by grants from the CICYT (SAF-99-0017) (Ministerio de Educación y Ciencia, Spain), Marató TV-3 (97-TV1009), Fundación Ramón Areces and Fundación La Caixa. N.C. was supported by Institut d'Investigacions Biomèdiques Augustí Pi i Sunyer (IDIBAPS).

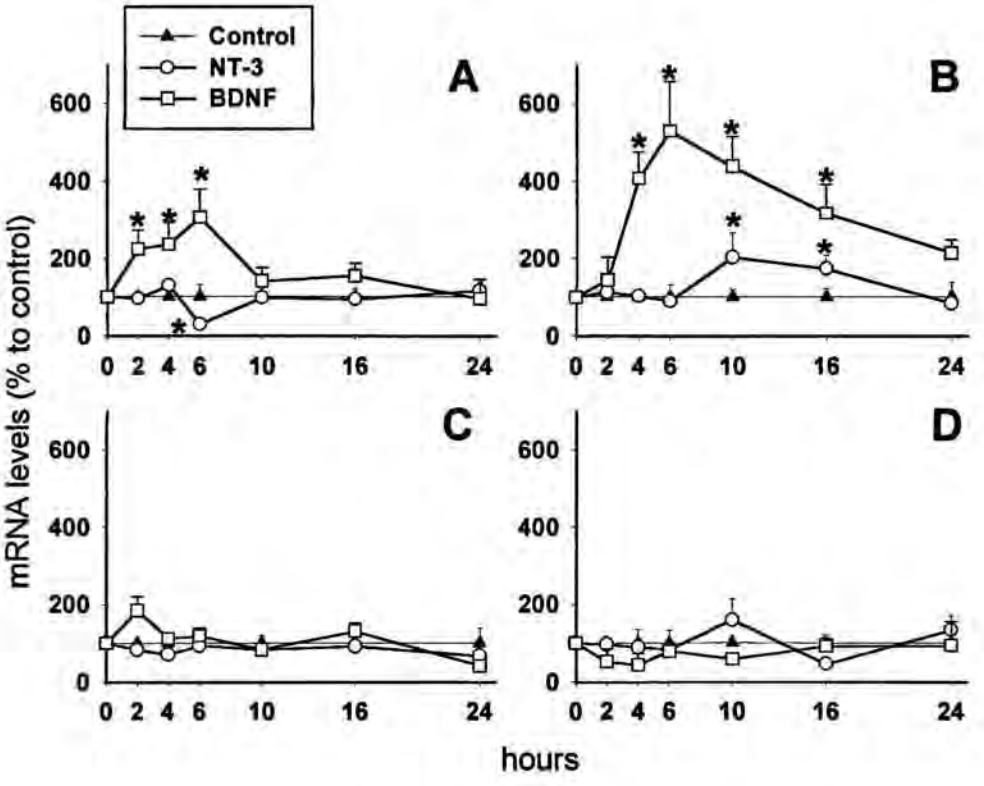

Figure 1. Time-course of BDNF and NT-3 mRNA levels in the ipsilateral cortex of glutamate receptor agonist-injected striata. QUIN (*A*), KA (*B*), AMPA (*C*) or ACPD (*D*) were injected into the striatum at two coordinates as indicated in Materials and Methods. QUIN upregulated BDNF expression but downregulated NT-3 (*A*). KA striatal injury produced upregulation of both BDNF and NT-3 mRNA levels (*B*). No significant changes in BDNF or NT-3 mRNA levels were observed in the cortex ipsilateral to AMPA- or ACPD-injected striata (*C*, *D*, respectively). Values are represented as mean ± S.E.M. (* $p<0.05$).

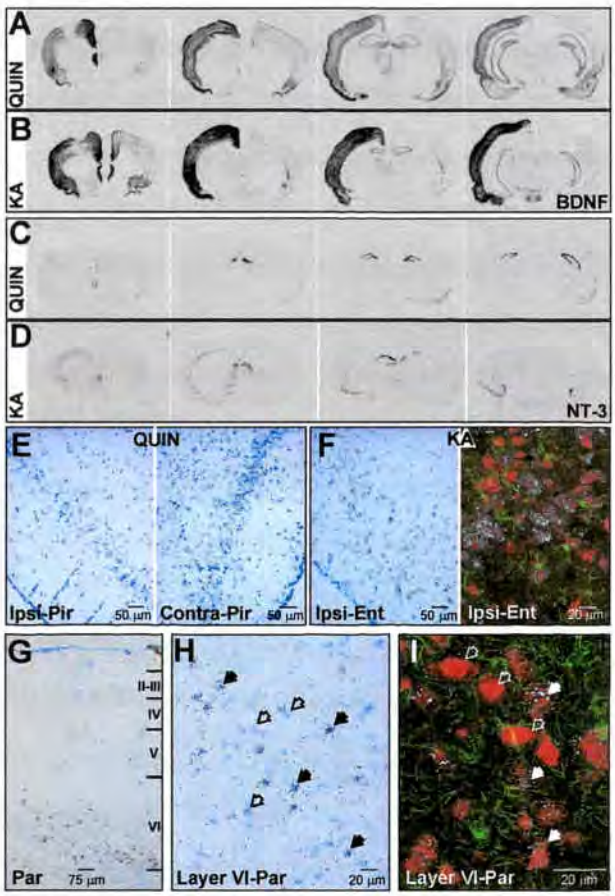

Figure 2. Changes in expression of BDNF and NT-3 are localized to neurons of different cortical areas following striatal injury with QUIN or KA. BDNF mRNA is upregulated in the cortex ipsilateral to excitotoxic lesion induced in the striatum by QUIN (*A*) or KA (*B*). High levels of BDNF expression were detected in all cortical areas of the lesioned hemisphere (*left hemisphere*; *A, B*). Although the levels of NT-3 were low in control cortex, reduced expression of NT-3 was observed in the piriform cortex after striatal QUIN-injection (*C, E*). In contrast, NT-3 expression increased in the cortex ipsilateral to KA-injected striata (*D, F-I*). This enhancement of NT-3 mRNA was mainly localized to the entorhinal cortex (*F*) and layer VI of the fronto-parietal cortex (*G, I*). NT-3 expression (*white*) was mainly localized to NeuN-positive cells (*red*) whereas GFAP-positive astrocytes (*green*) were negative (*F, I*). However, some cortical neurons upregulated NT-3 expression (*close arrows*) while others did not (*open arrows*; *H, I*).

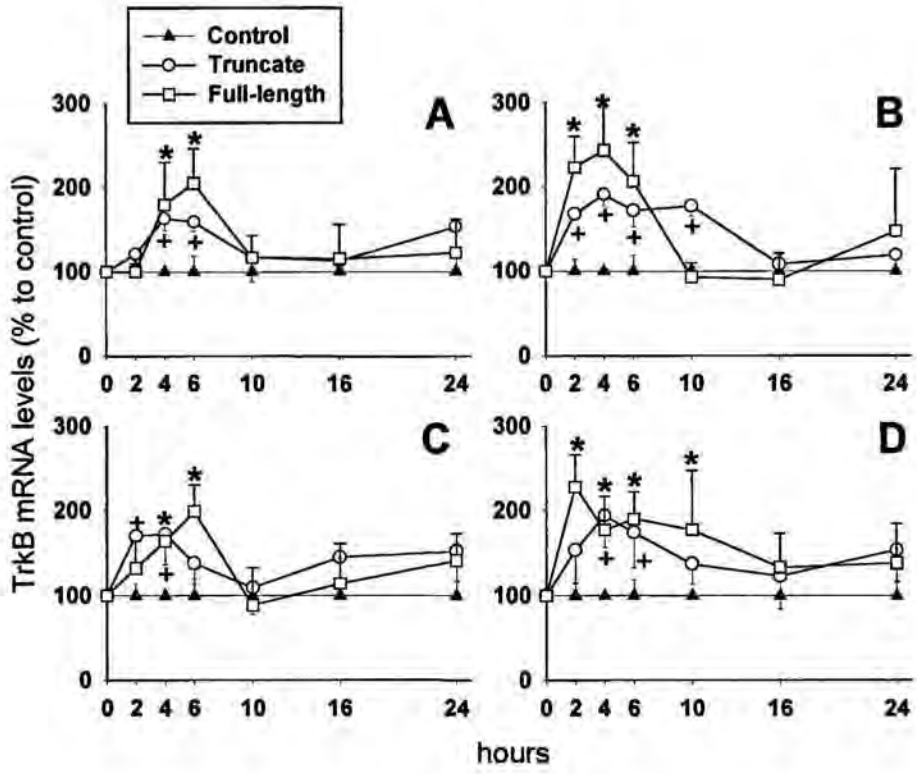

Figure 3. Both forms of TrkB receptor were upregulated in cortex following intrastriatal injection of QUIN (*A*), KA (*B*), AMPA (*C*) or ACPD (*D*). A probe that recognizes the two TrkB forms separately allowed us to characterize the expression of the catalytic and the truncated from of the receptor. The highest levels of full-length mRNA were observed in cortex after KA-striatal injury (*B*). However, the truncated form showed the largest cortical expression at 4 h in the ACPD injected animals. Values represent means ± S.E.M. (+ $p<0.05$ for *Truncate* and * $p<0.05$ for *Full-length* compare to control).

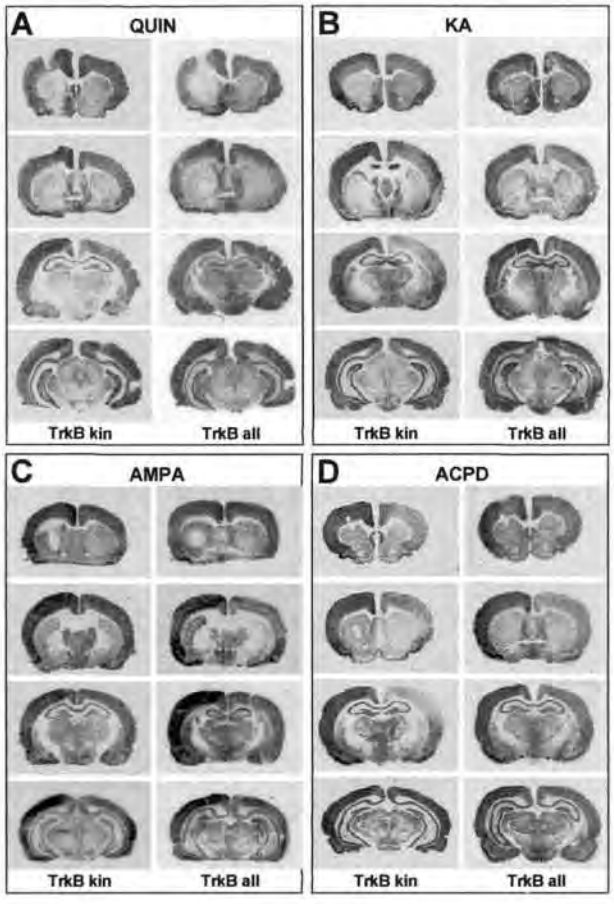

Figure 4. TrkB mRNA levels increased in the cortex ipsilateral to glutamate receptor agonist-injected striata with QUIN (A), KA (B), AMPA (C) and ACPD (D). Autoradiograms showing a 20-day exposure of coronal sections through brains receiving intrastriatal injection of glutamate receptor agonists. No regional differences were observed between the two probes used; one recognizes the full-length form (*TrkB kin*) and the other detects both isoforms of the TrkB receptor (*TrkB all*). The same pattern of TrkB expression was observed in all the conditions studied.

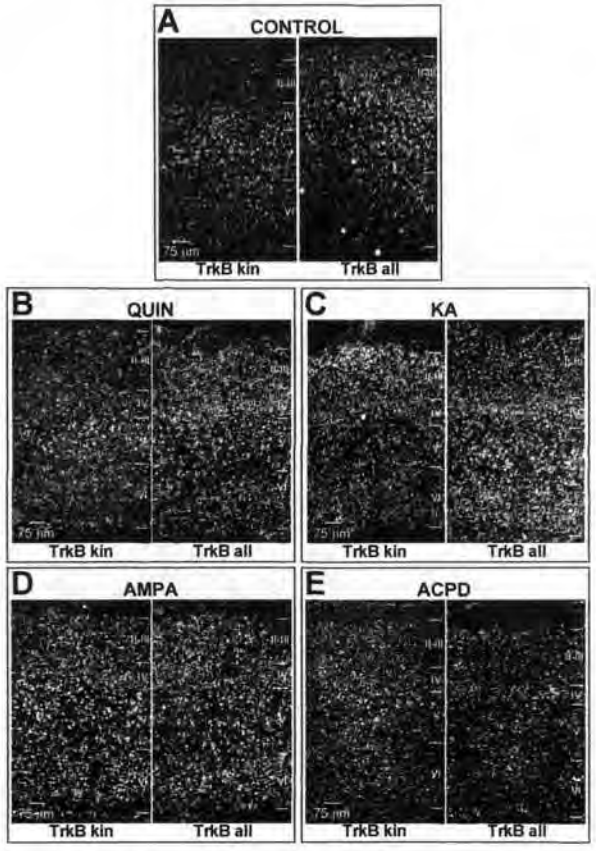

Figure 5. Distribution of truncated and full-length TrkB isoforms in cortical layers. Dark-field of *in situ* hybridization showed differences in TrkB-isoforms expression in cortical layers of sham injected animals (*A*). Using the probe that recognizes both truncated and full-length isoforms (*TrkB all*) we detected expression of TrkB receptor in layer IV, which was not detected with the probe that only recognize the full-length form (*TrkB kin; A*). The differences between receptor isoforms were more evident after intrastriatal injection of glutamate receptor agonist (*B-E*).

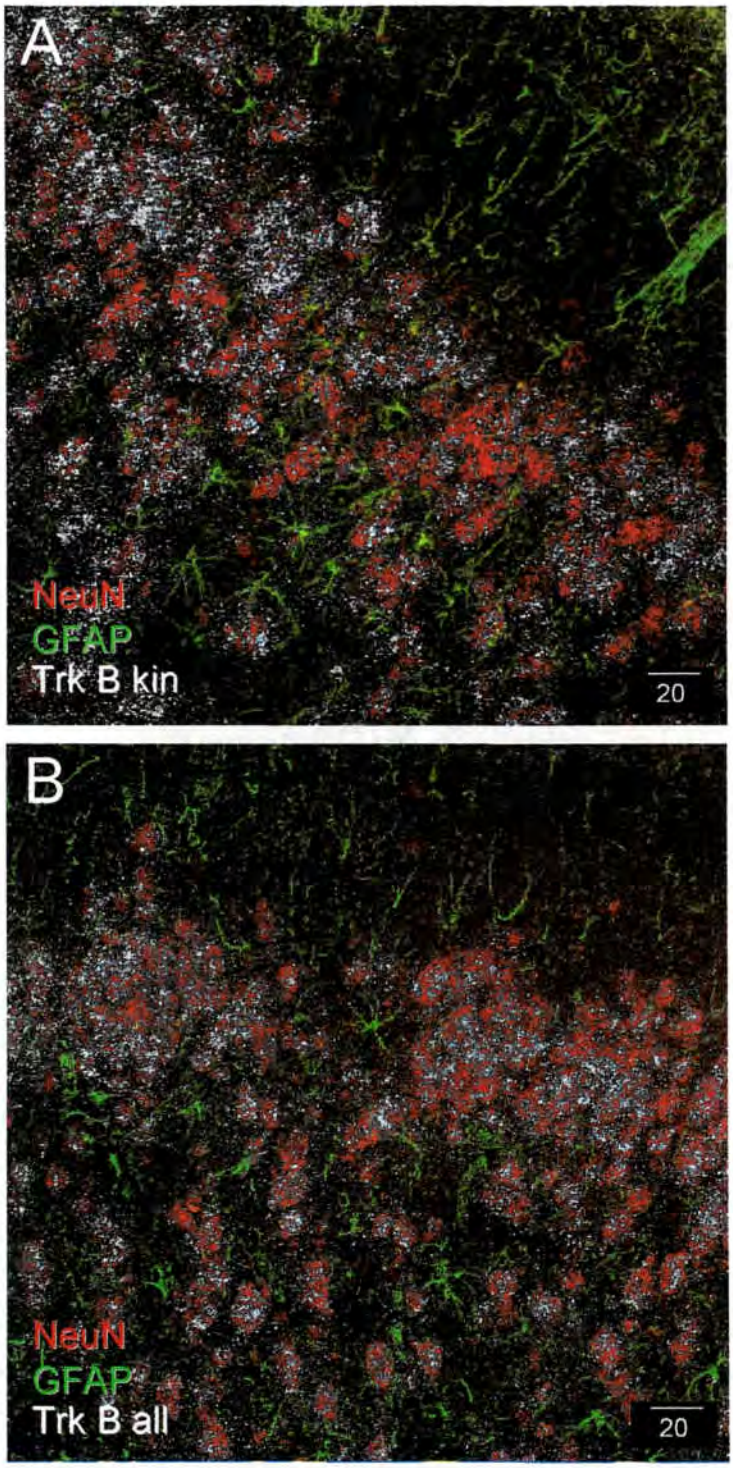

Figure 6. Both TrkB isoforms increased in neurons following striatal excitotoxic injury. Photomicrographs showing triple-labeling of cortical layer II-III. NeuN-positive neurons are labeled in red, GFAP-positive astrocytes are labeled in green, and white corresponds to the BDNF hybridization signal as assessed by radioactive hybridization *in situ*. Using a probe that only recognizes the full-length isoform of the BDNF receptor (*TrkB kin*), the increase in signal was localized to cortical neurons (*A*). In addition, using the probe that recognizes both receptor isoforms, enhanced TrkB expression was also detected in neurons (*TrkB all*; *B*).

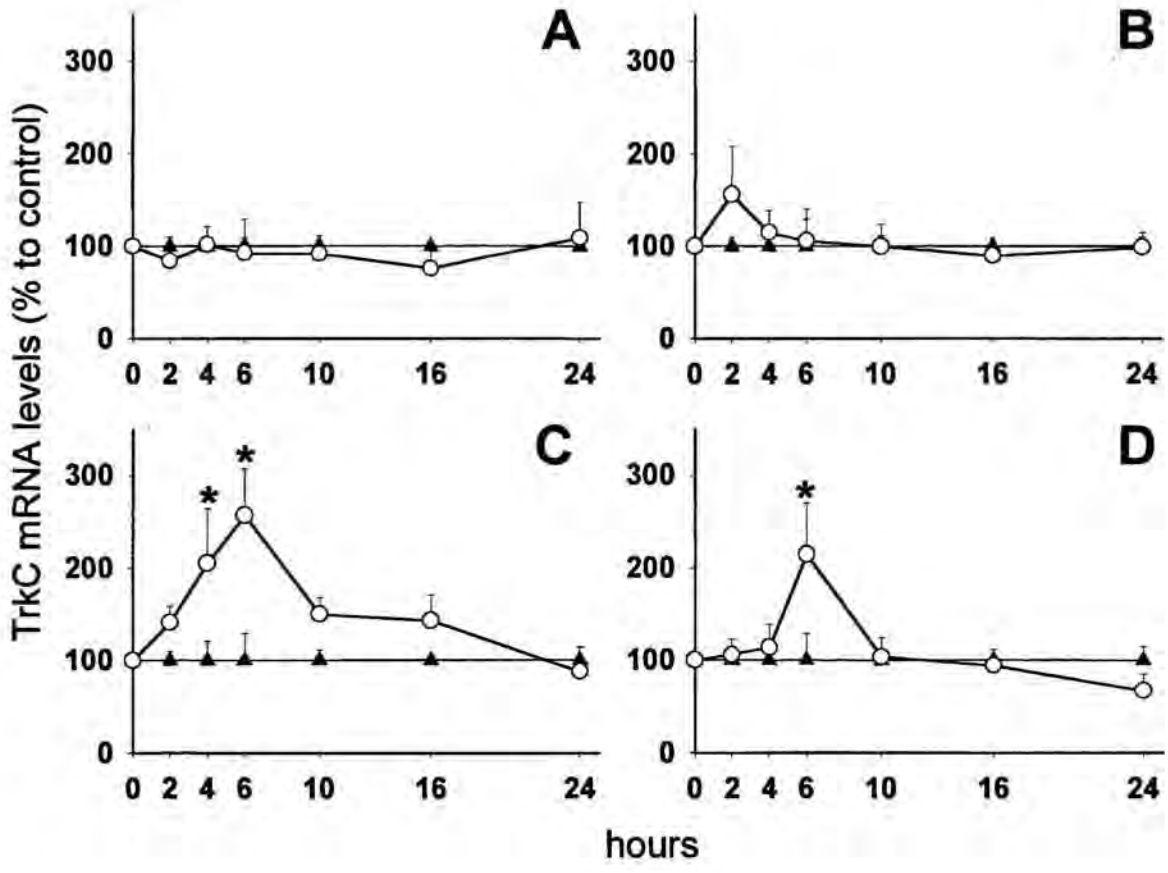

Figure 7. Changes of *Trk*C mRNA were complementary to the expression of its ligand, NT-3, in cortex after striatal injection of different glutamate receptor agonists. Thus, the glutamate receptor agonists that modified the levels of NT-3 mRNA, QUIN (*A*) and KA (*B*), did not change the expression of TrkC receptor. The highest changes in cortical TrkC expression were observed 6 h after intrastriatal injection of AMPA (*C*). High levels of this receptor mRNA were also observed in the ipsilateral cortex to ACPD-lesioned striata (D). Closed-triangles represent the data from sham-injected rats whereas open-circles represent the results from animals injected with glutamate receptor agonist. Values are represented as mean ± S.E.M. (* $p<0.05$).

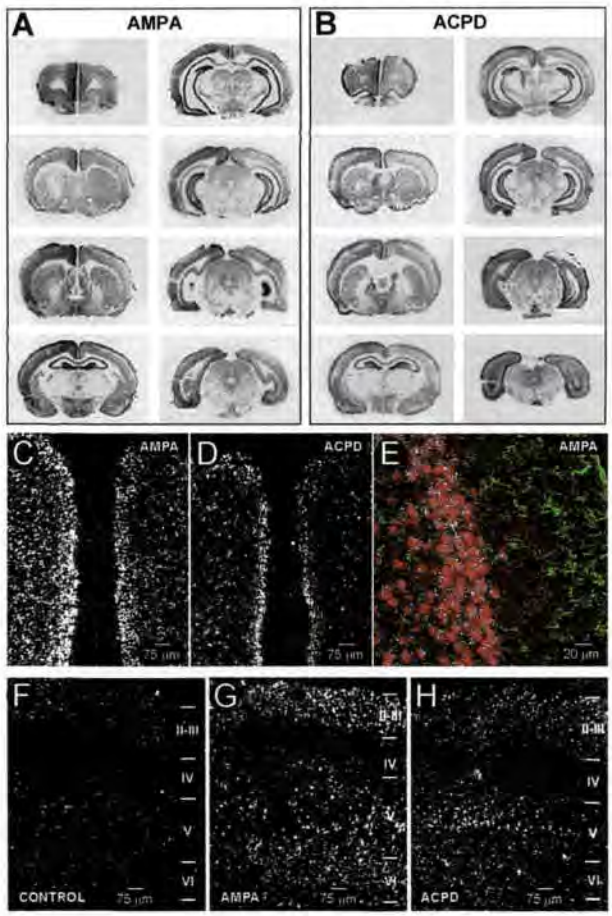

Figure 8. *In situ* hybridization showed changes of TrkC mRNA in cortical areas that project to the striatum. Expression of the receptor for NT-3 increased in the fronto-parietal, temporal and cingulate cortex (*A, B*). Dark-field showed high levels of TrkC expression in the cingulate area (*C, D*). The increase was higher in the ipsilateral cortex of AMPA-injected animals (*C*). In fronto-parietal and temporal areas the increase in TrkC expression was detected in layers II-III, V and VI (*F-H*). Triple labeling showed that the enhancement of TrkC expression was localized to neurons (*E*).

References

Alderson RF, Curtis R, Alterman AL, Lindsay RM, DiStefano PS (2000) Truncated TrkB mediates the endocytosis and release of BDNF and neurotrophin-4/5 by rat astrocytes and Schwann cells *in vitro*. Brain Res 871:210-222.

Altar A, DiStefano PS (1998) Neurotrophin trafficking by anterograde transport. Trends Neurosci 21:433-437.

Armanini MP, McMahon SB, Sutherland J, Shelton DL, Phillips HS (1995) Truncated and catalytic isoforms of trkB are co-expressed in neurons of rat and mouse CNS. Eur J Neurosci 7:1403-1409.

Barbany G, Persson H (1993) Adrenalectomy attenuates kainic acid-elicited increases of messenger RNAs for neurotrophins and their receptors in the rat brain. Neuroscience 54:909-922.

Baxter GT, Radeke MJ, Kuo RC, Makrides V, Hinkle B, Hoang R, Medina-Selby A, Coit D, Valenzuela P (1997) Signal transduction mediated by the truncated trkB receptor isoforms, trkB.T1 and trkB.T2. J Neurosci 17:2683-2690.

Beal MF, Ferrante RJ, Swartz KJ, Kowall NW (1991) Chronic Quinolinic acid lesions in rats closely resemble Huntington's disease. J Neurosci 11:1649-1659.

Bengzon J, Kokaia Z, Ernfors P, Kokaia M, Leanza G, Nilsson OG, Persson H, Lindvall O (1993) Regulation of neurotrophin and trkA, trkB and trkC tyrosine kinase receptor messenger RNA expression in kindling. Neuroscience 53:433-446.

Biffo S, Offenhauser N, Carter BD, Barde Y-A (1995) Selective binding and internalization by truncated receptors restrict the availability of BDNF during development. Development 121:2461-2470.

Bonhoeffer T (1996) Neurotrophins and activity-dependent development of the neocortex. Curr Opin Neurobiol 6:119-126.

Canals JM, Marco S, Checa N, Michels A, Pérez-Navarro E, Arenas E, Alberch J (1998) Differential regulation of the expression of NGF, BDNF and NT-3 after excitotoxicity in a rat model of Huntington's disease. Neurobiol Dis 5:357-364.

Canals JM, Checa N, Marco S, Michels A, Pérez-Navarro E, Alberch J (1999) The neurotrophin receptors trkA, trkB and trkC are differentially regulated after excitotoxic lesion in rat striatum. Mol Brain Res 69:242-248.

Canals JM, Checa N, Marco S, Åkerud P, Michels A, Pérez-Navarro E, Tolosa E, Arenas E, Alberch J (2001) Expression of brain-derived neurotrophic factor in cortical neurons is regulated by striatal target area. J Neurosci 21:117-124.

Castren E, da Penha Berzaghi M, Lindholm D, Thoenen H (1993) Differential effects of MK-801 on brain-derived neurotrophic factor mRNA levels in different regions of the rat brain. Exp Neurol 122:244-252.

Checa N, Canals JM, Alberch J (2000) Developmental regulation of BDNF and NT-3 expression by quinolinic acid in the striatum and its main connections. Exp Neurol 165:118-124.

Cheng B, Mattson MP (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. Brain Res 640:56-67.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Analyt Biochem 162:156-159.

Connor B, Dragunow M (1998) The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Rev 27:1-39.

Cudkowicz M, Kowall NW (1990) Degeneration of pyramidal projection neurons in Huntington's disease cortex. Ann Neurol 27:200-204.

de la Monte SM, Vonsattel JP, Richardson EP (1988) Morphometric demonstration of atrophic change in the cerebral cortex. J Neuropathol Exp Neurol 47:525-

DiFiglia M (1990) Excitotoxic injury of the neostriatum: a model for Huntington's disease. Trends Neurosci 13:286-289.

Eide FF, Vining ER, Eide BL, Zang K, Reichardt LF (1996) Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. J Neurosci 16:3123-3129.

Elmer E, Kokaia M, Kokaia Z, Ferencz I, Lindvall O (1996) Delayed kindling development after rapidly recurring seizures: relation to mossy fiber sprouting and neurotrophin, GAP-43 and dynorphin gene expression. Brain Res 712:19-34.

Ferrer I, Goutan E, Marin C, Rey MJ, Ribalta T (2000) Brain-derived neurotrophic factor in Huntington's disease. Brain Res 866:257-261.

Friedman WJ, Black IB, Kaplan DR (1998) Distribution of the neurotrophins brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 in the postnatal rat brain: an immunocytochemical study. Neuroscience 84:101-114.

Frisén J, Verge VM, Fried K, Risling M, Persson H, Trotter J, Hökfelt T, Lindholm D (1993) Characterization of glial trkB receptors: Differential response to injury in the central and peripheral nervous systems. Proc Natl Acad Sci USA 90:4971-4975.

Fryer RH, Kaplan DR, Kromer LF (1997) Truncate trkB receptors on nonneuronal cells inhibit BDNF-induced neurite outgrowth *in vitro*. Exp Neurol 148:616-627.

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285-320.

Giehl KM, Schütte A, Mestres P, Yan Q (1998) The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons *in vivo* is mediated by an endogenous brain-derived neurotrophic factor mechanism. J Neurosci 18:7351-7360.

Isackson PJ (1995) Trophic factor response to neuronal stimuli or injury. Curr Opin Neurobiol 5:350-357.

Kim HG, Wang T, Olafsson P, Lu B (1994) Neurotrophin 3 potentiates neuronal activity and inhibits GABA synaptic transmission in cortical neurons. Proc Natl Acad Sci USA 91:12341-12345.

Kokaia Z, Andsberg G, Yan Q, Lindvall O (1998) Rapid alterations of BDNF protein levels in the rat brain after focal ischemia: Evidence for increased synthesis and anterograde axonal transport. Exp Neurol 154:289-301.

Kume T, Kouchiyama H, Kaneko S, Maeda T, Akaike A, Shimohama S, Kihara T, Kimura J, Wada K, Koizumi S (1997) BDNF prevents NO mediated glutamate cytotoxicity in cultured cortical neurons. Brain Res 756:200-204.

Laurenzi MA, Barbany G, Timmusk T, Lindgren JA, Persson H (1994) Expression of mRNA encoding neurotrophins and neurotrophin receptors in rat thymus, spleen tissue and immunocompetent cells. Regulations of neurotrophin-4 mRNA expression by mitogens and leukotriene B4. Eur J Biochem 223:733-741.

Lindefors N, Brondin E, Metsis M (1995) Spatiotemporal selective effects on brain-derived neurotrophic factor and *trk*B messenger RNA in rat hippocampus by electroconvulsive shock. Neuroscience 65:661-670.

Lindvall O, Kokaia Z, Bengzon J, Elmer E, Kokaia M (1994) Neurotrophins and brain insults. Trends Neurosci 17:490-496.

Lodovichi C, Berardi N, Pizzorusso T, Maffei L (2000) Effects of neurotrophins on cortical plasticity: same or different? J Neurosci 20:2155-2165.

Marty S, Berzaghi MP, Berninger B (1997) Neurotrophins and activity-dependent plasticity of cortical interneurons. Trends Neurosci 20:198-202.

McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dentritic growth. Neuron 18:767-778.

McAllister AK, Katz LC, Lo DC (1999) Neurotrophins and synaptic plasticity. Annu Rev Neurosci 22:295-318.

Merlio JP, Ernfors P, Jaber M, Persson H (1992) Molecular cloning of rat trkC and distribution of cells expressing messenger RNA for members of the trk family in the rat central nervous system. Neuroscience 51:513-532.

Merlio JP, Ernfors P, Kokaia Z, Middlemas DS, Bengzon J, Kokaia M, Smith M-L, Siesjö BK, Hunter T, Lindvall O, Persson H (1993) Increased production of the TrkB protein tyrosine kinase receptor after brain insults. Neuron 10:151-164.

Middlemas DS, Lindberg RA, Hunter T (1991) trkB, a neural receptor protein-Tyrosine Kinase: evidence for a full-length and two truncated receptors. Mol Cell Biol 11:143-153.

Miranda RC, Sohrabji F, Toran-Allerand D (1993) Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. Proc Natl Acad Sci USA 90:6439-6443.

Parent A, Hazrati L-N (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Rev 20:91-127.

Pitts AF, Miller MW (2000) Expression of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 in the somatosensory cortex of the mature rat: coexpression with high affinity neurotrophin receptor. J Comp Neurol 418:241-254.

Reichardt LF, Fariñas I (1997) Neurotrophic factors and their receptors: roles in neuronal development and function. In: Molecular approaches to neuronal development (Cowan, M. W.),pp 220-263. New York: Oxford University Press

Rocamora N, Massieu L, Boddeke HWGM, Palacios JM, Mengod G (1994) Differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 mRNAs in adult rat brain after intrahippocampal injection of quinolinic acid. Mol Brain Res 26:89-98.

Rocamora N, Welker E, Pascual M, Soriano E (1996) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. J Neurosci 16:4411-4419.

Schmidt-Kastner R, Wetmore C, Olson L (1996) Comparative study of brain derived neurotrophic factor messenger RNA and protein at cellular level suggests multiple roles in hippocampus, striatum and cortex. Neuroscience 74:161-183.

Schütte A, Yan Q, Mestres P, Giehl KM (2000) The endogenous survival promotion of axotomized rat corticospinal neurons by brain-derived neurotrophic factor is mediated via paracrine, rather than autocrine mechanisms. Neurosci Lett 290:185-188.

Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627-638.

Takei N, Sasaoka K, Inoue K, Takahashi M, Endo Y, Hatanaka H (1997) BDNF increases the stimulation-evoked release of glutamate and the levels of excytosis-associated proteins in cutured cortical neurons from embryonic rats. J Neurochem 68:370-375.

Thoenen H (1995) Neurotrophins and neuronal plasticity. Science 270:593-598.

Xu B, Zang H, Ruff NL, Zhang YA, McConnell SK, Stryker MP, Reichardt LF (2000) Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron 26:233-245.

Yacoubian TA, Lo DC (2000) Truncated and full-length TrkB receptors regulate distinct modes of dendritic growth. Nat Neurosci 3:342-349.

Yuste R, Sur M (1999) Developmental and plasticity of the cerebral cortex: from molecules to maps. J Neurobiol 41:1-6.

ENDOGENOUS REGULATION OF BDNF EXPRESSION IN THE SUBSTANTIA NIGRA PARS COMPACTA AFTER STRIATAL EXCITOTOXIC INJURY

Article en preparació

ENDOGENOUS REGULATION OF BDNF EXPRESSION IN THE SUBSTANTIA NIGRA PARS COMPACTA AFTER STRIATAL EXCITOTOXIC INJURY

Anna M. Canudas[1,2], Susanna Pezzi[1], Josep M. Canals[1], Mercè Pallàs[2] and Jordi Alberch[1]

[1]Departament de Biologia Cel·lular i Anatomia Patològica, Facultat de Medicina, Universitat de Barcelona, IDIBAPS, Casanova 143, E-08036 Barcelona, Spain. [2]Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona, Nucli Universitari Pedralbes, Barcelona, Spain

Key words: basal ganglia, substantia nigra, neurotrophins, excitotoxicity, transneuronal

Running head: REGULATION OF NIGRAL BDNF EXPRESSION

Correspondence to: Jordi Alberch

Departament de Biologia Cel·lular i Anatomia Patològica. Facultat de Medicina.

Universitat de Barcelona. Casanova 143, E-08036 Barcelona, Spain.

Phone number: 34-3-4035285

FAX number: 34-3-4021907

E-mail: alberch@medicina.ub.es

ABSTRACT

Some neurons die after lesion of their afferents or efferents. This transneuronal degeneration takes place in several neurodegeneratives disordes amplifying the patHology. Excitotoxic lesions of the striatum have been shown to result in delayed death of neurons in the substantia nigra pars reticulata without neuronal loss in the pars compacta. Dopaminergic nigral cells synthesize BDNF and this neurotrophin can prevent the loss of nigral neurons after different insults. We have studied changes in the expression pattern of BDNF in the substantia nigra induced by intrastriatal injection of kainic acid. Our results show an increased levels of this neurotrophin in the pars compacta after intrastriatal lesion. When BDNF was blocked by infusion of TrkB bodies, TH positive cells showed an atrophy.

These results demonstrate that endogenous regulation of BDNF protects substantia nigra pars compacta neurons from their trophic target loss.

INTRODUCTION

Transneuronal degeneration is observed in some groups of neurons following damage to their afferents or efferents (Cowan, 1970). This pathological process may play a role in several neurodegenerative disorders, such as Huntington's chorea or Parkinson's disease. In experimental models of these disorders, it has been reported an atrophy or death of substantia nigra neurons following excitotoxic or ischemic damage to the striatum (Saji et al., 1986, 1993, 1994; Volpe et al., 1995). However, nigral neurons show different vulnerability. Excitotoxic lesions in the striatum and the globus pallidus induce an anterograde transneuronal degeneration of neurons in the substantia nigra pars reticulata (Saji et al., 1993). However, striatal lesions do not affect retrogradely dopaminergic neurons in the substantia nigra pars compacta. Imbalance between excitatory and inhibitory inputs within neural circuits after striatal lesion may be a cause of secondary degeneration of neurons. The loss of nigral neurons can be prevented by ablation of their glutamatergic input from the subthalamic nucleus (Saji et al., 1996), by infusion of the GABAergic agonist muscimol (Saji et al., 1986) or by administration of glutamatergic antagonists (Yamada et al., 1996, DeGiorgio et al., 1999). Thus, acceleration of excitatory input from the dishibited subthalamic nucleus and deprivation of inhibitory input from the striatum can induce the degeneration of neurons of the substantia nigra pars reticulata.

The trophic response of each neuronal population may also be involved in the regulation of the differential vulnerability against different insults. Furthermore, changes in neuronal activity modify the expression of neurotrophic factors and their receptors (Mundo et al., 1996). BDNF has been described as an important regulator of the maintenance of normal function of dopaminergic neurons in the adult substantia nigra. Moreover, this neurotrophin exerts a neuroprotective effect on nigrostriatal dopaminergic neurons (Hyman et al., 1994; Altar et al., 1994) and GABAergic striatonigral and striatopallidal neurons (Arenas et al., 1996; Altar et al., 1994) against a number of neurotoxic agents. It has also been reported that BDNF prevents the transneuronal degeneration of nigral neurons induced by excitotoxic striatal-pallidal lesions (Volpe et al., 1998). On the other hand, BDNF participates in the induction of dopaminergic sprouting after striatal lesion (Batchelor et al., 2000).

In previous studies, it has been reported a transneuronal regulation of neurotrophins expression following different stimuli. Thus, BDNF is regulated in cortical neurons after striatal lesion, suggesting a autocrine/paracrine protective effect against the removal of their target (Canals, 2001). Therefore, the aim of the present study is to evaluate changes in the expression of BDNF in the substantia nigra and its involvement of different populations of the pars compacta and pars reticulata.

MATERIAL AND METHODS

Animal treatment. Male Sprague-Dawley rats (140-210 gr) were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a David Kopf stereotaxic apparatus (DK 900) with the incisor bar 5 mm above the interaural line. kainate (KA, 6 nmol; Sigma) and phosphate buffered saline (PBS; pH, 7.4) were injected in a volume of 1 µl into the left neostriatum at two coordinates (AP, +2.3 and L, -2.4 from bregma, and - 5.0 from dura; AP, +1.3 and L, -3.0 from bregma, and - 5.0 from dura) as previously described (Canals et al., 1998). Trk B body assay: before the intraestriatal injection, a 6.5 mm long, 28-gauge stainless steel cannula (Plastics One, Roanoke, VA.) was mounted on the skull with cyanoacrylate adhesive at a location on the skull that placed the cannula tip above the left substantia nigra (coordinates: AP, - 2.2 and L, - 1.9 from bregma, and – 6.2 from dura). The metal inlet of the cannula was fitted to a 10 cm length of silastic PE50 tubing (Micro-Renathane. Braintree. MA). The tubbing was filled with the same solution that was placed in the osmotic pump (model 1002; Alza Co., Palo Alto, CA) and attached to the other end of the tubing. The Alzet pump was filled immediately prior to the surgery with 6 µl of the Trk B body or IgG (3.33 µg/µl) The pump flow rate was 0.25 µl/h. The pump was implanted into a subcutaneous space between the shoulder blades that was created with a haemostat inserted caudally from the scalp wound.

After lesioning, animals were housed separately with access to food and water *ad libitum* in a colony room maintained at constant temperature (19-22°C) and humidity (40-50%) on a 12:12 h light/dark cycle. All animal-related procedures were in

accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals and approved by the local animal care committee (C.E.E.A.; Comitè Ètic d'Experimentació Animal) of the Universitat de Barcelona (99/01) and by the Generalitat de Catalunya (99/1094).

RNAse protection assay. Rats were killed by decapitation at 2, 4, 6, 10, 16, 24 or 72 h after kainic acid injection, injury, and brains were removed and substantia nigra (including pars compacta, pars reticulata and ventral tegmental area) were quickly dissected out, frozen on dry ice, and stored at –80°C . Total RNA was isolated by the guanidine isothiocyanate method (Chomczynski and Sacchi, 1987). The assay was performed as described by the manufacturer. Antisense cRNA probes for BDNF and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were prepared as previously described (Canals i col., 1998). All probes were labeled by *in vitro* transcription using T7 RNA polymerase (Promega, Madison, WI) and [α-^{32}P] CTP (Amersham Intl., Little Chalfont, UK). In each reaction, total RNA (30 µg) was hybridized at 42°C for 16 h to one of the neurotrophin antisense cRNA probes together with the GAPDH cRNA probe in order to standardize the amount of mRNA (Fort et al., 1985). Protected fragments were separated on 4% polyacrylamide gels under denaturing conditions, and gels were exposed to X-OMAT (Kodak, Rochester, NY, USA) films at -80°C with an intensifying screen. The optical density of the autoradiograms was measured and quantified with the Phoretix 1D gel analysis (Phoretix International Ltd., Newcastle, UK).

In situ *hybridization studies.* Animals were killed 16 h after intrastriatal injection of PBS or kainic acid. Brains were dissected out and frozen in dry-ice cooled isopentane. Serial coronal cryostat sections (14 µm) were processed for hybridization with radioactive BDNF riboprobe (Lindefors et al., 1995) as follows. After rinsing in PBS, tissue was deproteinated in 0.2 M HCl for 10 min, acetylated with 0.25% acetic anhydride in 0.1 M ethanolamine for 10 min, postfixed in 4% paraformaldehyde and dehydrated with increasing concentrations of ethanol. Slices were incubated for 16 h in a humidified chamber at 53°C with 3 x 10^5 cpm of antisense BDNF probe in 150 µl of hybridization cocktail (50% formamide, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0,

0.3 M NaCl, 0.1 M dithiothreitol, 0.5 mg/ml yeast tRNA, 0.1 mg/ml polyA RNA, 1x Denhardt's solution, and 10% dextran sulfate). Antisense cRNA probe to detect the BDNF transcript was prepared by *in vitro* transcription using T7 RNA polymerase (Promega, Madison, WI) and [^{35}S]- or [^{32}P]-UTP (Amersham Pharmacia Biotech, Upsala, Sweden). For control experiments, sense cRNA probe was obtained by *in vitro* transcription using T3 RNA polymerase (Promega). After hybridization, slices were first washed at room temperature in 1x SSC followed by two washes in 1x SSC at 37°C for 15 min each. Single-stranded RNA was digested by RNase treatment (40 µg/ml) for 30 min at 37°C in 0.5 M NaCl, 20 mM Tris-HCl, pH 7.6, 2 mM EDTA. Tissue was washed twice in 1x SSC in 65°C for 10 min and then dehydrated in ethanol and air-dried. Slices were exposed to β-max x-ray film (Amersham Pharmacia Biotech) for 20 days and dipped in LM-1 photoemulsion (Amersham Pharmacia Biotech), exposed at 4°C for 2 months, developed with D19 (Kodak), fixed and counterstained with cresyl violet staining.

Statistical analysis. The results were normalized to the mean of sham-injected animals (100%) and expressed as a percentage of these data. In our experimental conditions, we did not observe any differences in neurotrophin expression between sham and non-injected animals. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by LDS post-hoc test.

TH and parvalbumine immunohistochemistry. Sections obtained with a cryostat (40 µm) were collected in PBS as free-floating sections and preincubated for 1 h with PBS containing 10% methanol and 3% H_2O_2. Sections were then washed three times in PBS, permeabilized with 0.5% Triton X-100 (Sigma) and preincubated for 2 h with 1.5% horse serum in PBS. After three washes in 0.1% Triton X-100 in PBS, tissue was incubated with the primary anti-TH antibody (1/1500; DiaSorin) and anti-parvalbumine (1/170; Sigma) in PBS containing 0.1% Triton X-100 and 1.5% horse serum for 16 h at 4°C. Sections were washed three times as above and incubated with a biotinylated rabbit anti-mouse antibody (1/170; Vector laboratories) for 1-2 h at room temperature in the

same buffer as the primary antibody. The immunohistochemical reaction was developed using the Vectastain ABC kit (Vector Laboratories).

RESULTS

Effect of striatal kainic acid lesion in BDNF expression in the substantia nigra

Using a RNase riboprotection assay, we evaluated the time course changes in nigral BDNF expression after intrastriatal injection of kainic acid. Our results show that mRNA levels of BDNF increased in the substantia nigra 4 hours following after striatal excitotoxic lesion (fig. 1). This enhancement was about 250% and it was maintained until 24 h after the injury.

The cellular localization of BDNF expression in the substantia nigra was detected by *in situ* hybridization. BDNF expression was low in control animals, and it was mainly located in the substantia nigra pars compacta. However, after striatal excitotoxic injury the levels of expression highly increase in the substantia nigra pars compacta (fig. 2). No enhancement was observed in the substantia nigra pars reticulata.

Differential vulnerability of neurons of substantia nigra pars compacta and pars reticulata following excitotoxic injury in the striatum

We have analyzed the effect of kainic acid injection in the striatum upon the different neuronal populations of the substantia nigra pars compacta and pars reticulata using immunohistochemistry. Tyrosine hydroxylase (TH) staining was used for dopaminergic neurons of the pars compacta, while neurons of the pars reticulata were labeled with antibodies against parvalbumin. Our results show the cell number of TH positive cells was not modified one week or even two weeks after the striatal lesion (fig. 3A). However, a delayed cell death in the substantia nigra pars reticulata following excitotoxic lesion in the striatum was observed (fig. 3B). A decrease (40%) of the parvalbumin-positive cells was detected 14 days after the lesion.

Effect of trkB bodies in the survival of neurons in the substantia nigra

To study the functional effect of the changes in BDNF expression, we injected trkB bodies in the substantia nigra, which selectively blocked the effect of endogenous BDNF. In control animals IgG was injected in the substantia nigra after striatal PBS or kainic injection. Our results showed that the injection of trkB bodies produced an atrophy of TH-positives cells (Fig. 4), but it did not modify parvalbumin-positive cells (data not shown). The morphological analysis showed that the volume of the substantia nigra pars compacta was reduced after the injection of the trkB bodies. The injection of IgG did not modify significantly any parameter analyzed. Furthermore the number of TH-positive cells was also decreased (20%) and they were smaller (fig. 4).

DISCUSSION

The present results show an increase of BDNF expression in the substantia nigra in response to kainic excitotoxic injury in the striatum. The neuronal population of substantia nigra show a differential vulnerability against striatal lesion. Thus, the number of parvalbumin-positive neurons of the pars reticulata were decreased, meanwhile the TH-positive cells of the pars compacta were spared. However, blocking endogenous BDNF with trk-IgG induced an atrophy of dopaminergic neurons of the substantia nigra pars compacta.

The transient increase in BDNF mRNA observed represents early changes to protect dopaminergic neurons in response to the excitotoxic lesion in the striatum. Previous studies have already shown that this type of lesion induces a local response enhancing BDNF expression in the striatum (Canals et al., 1998). Furthermore, the partial lesion of the nigrostriatal pathway by 6-hydroxydopamine in the striatum also induces an increase in BDNF mRNA expression in the lateral subregion of the substantia nigra pars reticulata (Aliaga et al., 2000). Moreover, BDNF protein levels also increase in the ventral mesencephalon of adult rats lesioned with 6-hydroxydopamine (Zhou et al., 1996). Hence, it is likely that non-dopaminergic neurons of the pars reticulata play a paracrine trophic role to dopaminergic neurons of the pars compacta. This transneuronal trophic regulation has been described in other systems. Striatal excitotoxic injury

produces an increase in BDNF expression in the cortical layers that project to the striatum, and this regulation is dependent of the striatal damage (Canals et al., 2001). Furthermore, corticospinal neurons are also protected of axotomy by increasing endogenous BDNF in cortical neurons (Giehl et al., 1998). Similarly, our results show that the lesion of the target area of the dopaminergic nigral neurons did not affect the integrity of these cells because of the increase in BDNF expression, showing an autocrine/paracine trophic support for nigral cells of the substantia nigra pars compacta. This response was specific for this neuronal population, since parvalbumin-positive cells of the pars reticulata were not affected.

Previous studies have shown that BDNF exerts a neuroprotective effect on nigrostriatal dopaminergic neurons against different types of insults (Hyman et al., 1991; Hyman et al., 1994; Spina et a., 1992). On the other hand, nigral infusions of BDNF also prevent the delayed, transneuronal degeneration of nigral neurons induced by excitotoxic striatal-pallidal lesions (Volpe et al., 1998). This nigral neuron degeneration can also be neutralized by glutamate receptor antagonists (DeGiorgio et al., 1999) or by subthalamic nucleus ablation (Saji et al., 1996). Thus, the neuronal loss in the substantia nigra is induced by excessive glutamatergic stimulation. Therefore, BDNF can be the neuroprotective agent from glutamatergic hyperactivity. In fact, this neurotrophin is able to protect neurons against excitotoxic insults in different systems (Cheng and Mattson, 1994; Shimohama et al., 1993; Perez-Navarro et al., 2000).

The neuroprotective mechanisms of BDNF is not well understood, although some evidences are already present. BDNF elevates calcium binding proteins in cultured neurons (Ip et al., 1993), that could buffer the toxic effect of intracellular calcium enhancement. Moreover, excitotoxicity also produces oxidative stress, and BDNF can reduce this effect increasing the mitochondrial enzyme glutathione reductase in cultured nigral neurons (Spina et al., 1992).

In conclusion, our results show that excitotoxic striatal lesion induced a transneuronal degeneration in the substantia nigra. However, the effect in the pars compacta can be counteract by increasing endogenous BDNF. Therefore, these results show that specific neuronal populations increase their trophic requirements to protect themselves against different insults.

ACKNOWLEDGEMENTS

We are very grateful to Mayte Muñoz for technical assistance. Financial support was obtained from CICYT (SAF96-0043; SAF99-0019; Ministerio de Educación y Ciencia, Spain), Fundació La Marató de TV3 (97-TV1009), La Fundació La Caixa y la Fundación Ramón Areces.

Figure 1

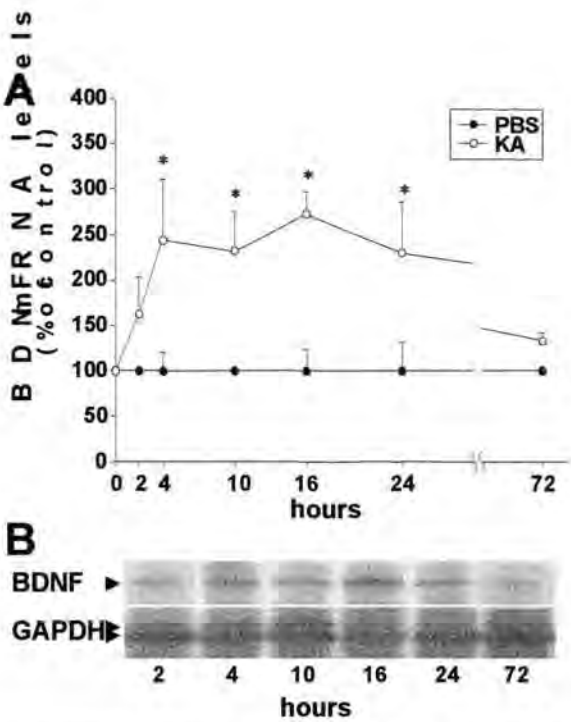

Fig 1.-Time-course of BDNF mRNA regulation in the ipsilateral substantia nigra of kainic acid injected striata. BDNF mRNA levels increased between 4 and 24 h after striatal lesion. Values are represented as mean ±SEM (*P<0.05) (A). RNAse protection representative experiment, protected fragments for BDNF and GAPDH (B).

Figure 2

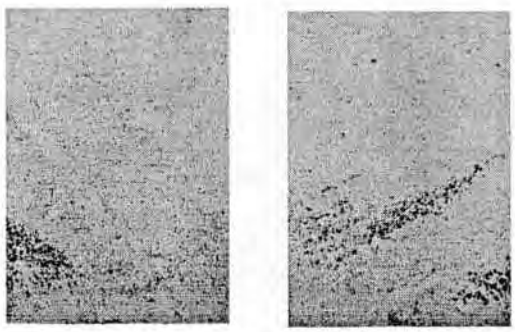

Fig 2.-*In situ* hibridization analysis showed increased levels of BDNF mainly located in the substantia nigra pars compacta. Contralateral (A) and ipsilateral (B) substantia nigra 16 h after striatal KA injection.

Figure 3

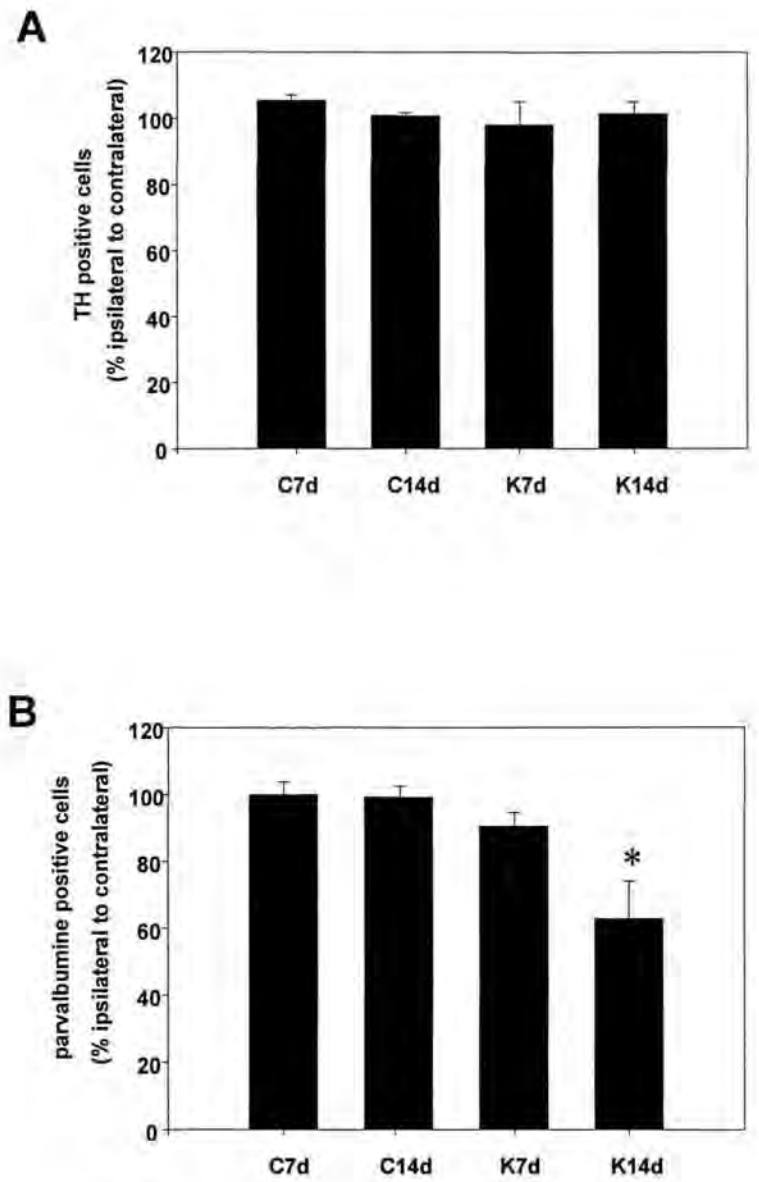

Fig 3.-The number of TH-positive cells are not modified one week or two weeks after the striatal lesion. Parvalbumine staining show a reduction 14 days after the lesion. Values are represented as mean ±SEM (*P<0.05)

Figure 4

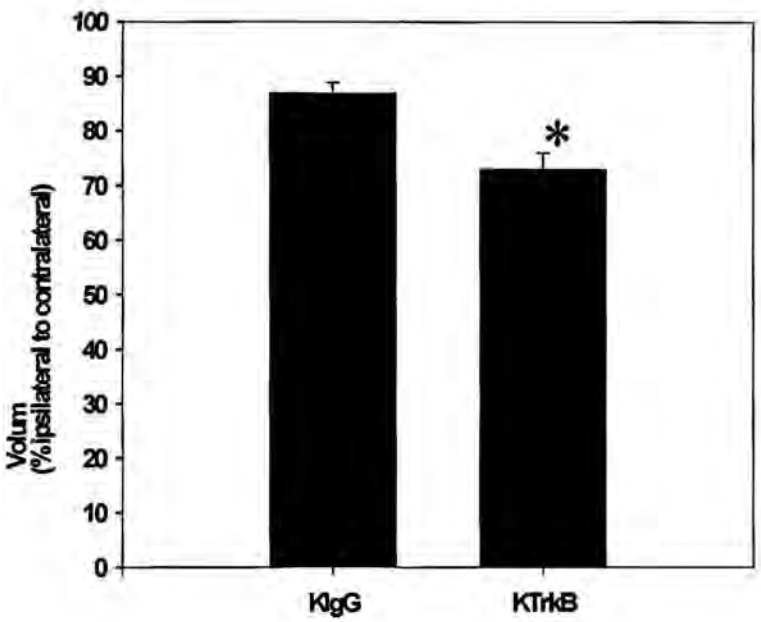

Fig 4.- Effect of Trk B bodies on ipsilateral substantia nigra volum after intrastrial kainic acid injection. Percentage of substantia nigra ipsilateral volum compared to contralateral side. Values are represented as mean ±SEM (*P<0.05)

BIBLIOGRAFIA

Aliaga, E., Carcamo, C., Abarca, J., Tapia-Arancibia, L. & Bustos, G. Transient increase of brain derived neurotrophic factor mRNA expression in substantia nigra reticulata after partial lesion of the nigrostriatal dopaminergic pathway. *Brain Res Mol Brain Res 2000. Jun. 23. ;79. (1. -2.):150. -5.*, **79**, 150-155.

Altar, C.A., Boylan, C.B., Fritsche, M., Jackson, C., Hyman, C. & Lindsay, R.M. (1994) The neurotrophins NT-4/5 and BDNF augment serotonin, dopamine, and GABAergic systems during behaviorally effective infusions to the substantia nigra. *Exp Neurol*, **130**, 31-40.

Altar, C.A., Boylan, C.B., Fritsche, M., Jones, B.E., Jackson, C., Wiegand, S.J., Lindsay, R.M. & Hyman, C. (1994) Efficacy of brain-derived neurotrophic factor and neurotrophin-3 on neurochemical and behavioral deficits associated with partial nigrostriatal dopamine lesions. *J Neurochem*, **63**, 1021-1032.

Arenas, E., Akerud, P., Wong, V., Boylan, C., Persson, H., Lindsay, R.M. & Altar, C.A. (1996) Effects of BDNF and NT-4/5 on striatonigral neuropeptides or nigral GABA neurons in vivo. *Eur J Neurosci*, **8**, 1707-1717.

Batchelor, P.E., Liberatore, G.T., Porritt, M.J., Donnan, G.A. & Howells, D.W. (2000) Inhibition of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor expression reduces dopaminergic sprouting in the injured striatum. *Eur J Neurosci* , **12**, 3462-3468.

Canals, J.M., Checa, N., Marco, S., Akerud, P., Michels, A., Perez-Navarro, E., Tolosa, E., Arenas, E. & Alberch, J.(2001) Expression of brain-derived neurotrophic factor in cortical neurons is regulated by striatal target area. *J Neurosci*, **21**, 117-124.

Canals, J.M., Marco, S., Checa, N., Michels, A., Perez-Navarro, E., Arenas, E. & Alberch, J. (1998) Differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 after excitotoxicity in a rat model of Huntington's disease. *Neurobiol Dis*, **5**, 357-364.

Cheng, B. & Mattson, M.P. (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. *Brain Res*, **640**, 56-67.

Chomczynski, P. & Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*, **162**, 156-159.

DeGiorgio, L.A., Dibinis, C., Milner, T.A., Saji, M. & Volpe, B.T. (1998) Histological and temporal characteristics of nigral transneuronal degeneration after striatal injury. *Brain Res*, **795**, 1-9.

DeGiorgio, L.A., DeGiorgio, N. & Volpe, B.T. (1999) Dizocilpine maleate, MK-801, but not 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline, NBQX, prevents transneuronal degeneration of nigral neurons after neurotoxic striatal-pallidal lesion. *Neuroscience,* **90**, 79-85.

Dluzen, D.E., Story, G.M., Xu, K., Kucera, J. & Walro, J.M. (1999) Alterations in nigrostriatal dopaminergic function within BDNF mutant mice. *Exp Neurol,* **160**, 500-507.

Giehl, K.M., Schutte, A., Mestres, P. & Yan, Q. (1998) The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons in vivo is mediated by an endogenous brain-derived neurotrophic factor mechanism. *J Neurosci,* **18**, 7351-7360.

Hyman, C., Juhasz, M., Jackson, C., Wright, P., Ip, N.Y. & Lindsay, R.M. (1994) Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *J Neurosci,* **14**, 335-347.

Ip, N.Y., Li, Y., Yancopoulos, G.D. & Lindsay, R.M. (1993) Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. *J Neurosci,* **13**, 3394-3405.

Mudo, G., Jiang, X.H., Timmusk, T., Bindoni, M. & Belluardo, N. (1996) Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. *Epilepsia,* **37**, 198-207.

Perez-Navarro, E., Canudas, A.M., Akerund, P., Alberch, J. & Arenas, E. Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 prevent the death of striatal projection neurons in a rodent model of Huntington's disease. *J Neurochem 2000. Nov. ;75. (5.):2190. -9.,* **75**, 2190-2199.

Saji, M. & Reis, D.J. (1987) Delayed transneuronal death of substantia nigra neurons prevented by gamma-aminobutyric acid agonist. *Science,* **235**, 66-69.

Saji, M. & Volpe, B.T. (1993) Delayed histologic damage and neuron death in the substantia nigra reticulata following transient forebrain ischemia depends on the extent of initial striatal injury. *Neurosci Lett,* **155**, 47-50.

Saji, M., Cohen, M., Blau, A.D., Wessel, T.C. & Volpe, B.T. (1994) Transient forebrain ischemia induces delayed injury in the substantia nigra reticulata: degeneration of GABA neurons, compensatory expression of GAD mRNA. *Brain Res,* **643**, 234-244.

Saji, M., Kimura, M., Ishida, G. & Ohno, K. (1995) Deafferentiation-induced c-fos gene expression in subthalamic nucleus and substantia nigra reticulata is reduced by non-NMDA receptor antagonist. *Brain Res,* **703**, 165-174.

Saji, M., Blau, A.D. & Volpe, B.T. (1996) Prevention of transneuronal degeneration of neurons in the substantia nigra reticulata by ablation of the subthalamic nucleus. *Exp Neurol,* **141**, 120-129.

Saji, M., Endo, Y., Miyanishi, T., Volpe, B.T. & Ohno, K. (1997) Behavioral correlates of transneuronal degeneration of substantia nigra reticulata neurons are reversed by ablation of the subthalamic nucleus. *Behav Brain Res,* **84**, 63-71.

Shimohama, S., Tamura, Y., Akaike, A., Tsukahara, T., Ohara, O., Watanabe, S. & Kimura, J. (1993) Brain-derived neurotrophic factor pretreatment exerts a partially protective effect against glutamate-induced neurotoxicity in cultured rat cortical neurons. *Neurosci Lett,* **164**, 55-58.

Spina, M.B., Squinto, S.P., Miller, J., Lindsay, R.M. & Hyman, C. (1992) Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J Neurochem,* **59**, 99-106.

Volpe, B.T., Wildmann, J. & Altar, C.A. (1998) Brain-derived neurotrophic factor prevents the loss of nigral neurons induced by excitotoxic striatal-pallidal lesions. *Neuroscience,* **83**, 741-748.

Yamada, K., Goto, S., Yoshikawa, M., Okamura, A. & Ushio, Y. (1997) Continuous intraventricular drug infusion for the in vivo study of transneuronal degeneration in the striatonigral system of the rat. *Brain Res Brain Res Protoc.,* **1**, 371-377.

Zhou, J., Pliego-Rivero, B., Bradford, H.F. & Stern, G.M. (1996) The BDNF content of postnatal and adult rat brain: the effects of 6-hydroxydopamine lesions in adult brain. *Brain Res Dev Brain Res,* **97**, 297-303.

DISCUSSIÓ

En les malalties degeneratives s'observa una resposta endògena tant a nivell neuronal com a nivell de les cèl·lules glials. L'estudi per el desenvolupament de nous tractaments per prevenir o aturar la progressió de la malaltia són de gran interés. En aquesta memòria es presenten diferents treballs experimentals que han tingut com a objectiu carateritzar i estudiar la resposta glial, així com la resposta tròfica i els mecanismes de neuroprotecció dels factors neurotròfics en models experimentals de malalties degeneratives dels ganglis basals com són la malaltia de Parkinson i la corea de Huntington.

Evaluació de la resposta glial en el nucli estriat per la injecció de l'MPP^+ en la substància negra.

En el primer treball presentat en aquesta memòria el model experimental utilitzat va ser la injecció de l'MPP^+ en la substància negra de rata, a fi d'estudiar la resposta glial secundària present en el nucli nucli estriat ipsilateral a la lesió. La injecció de l'MPP^+ produeix una lesió citotòxica a les neurones dopaminèrgiques, associada a una pèrdua de la dopamina estriatal (Sun i col., 1988; Neafsey i col., 1995). En el nostre estudi vam observar, en el nucli estriat ipsilateral a la lesió, un reducció del número de transportadors de dopamina a les diferents dosis d'MPP^+ utilitzades, quantificat mitjançant assatjos de unió amb GBR-12935 tritiat, i a més a més una disminució de la immunoreactivitat per la tirosina hidroxilasa, resultats que en demostraven una pèrdua de la innervació dopaminèrgica en el nucli estriat.

Les alteracions observades en els terminals dopaminèrgics anaven acompanyades per un increment en el nombre de receptors benzodiacepínics perifèrics, receptors que estan associats principalment a la glia (Anholt i col., 1984; Bender y col., 1987; Gehlert y col., 1985) i per això són molt utilizats com a marcadors de la reactivitat glial (Anholt i col., 1984; Benavides i col., 1990, 1991; Myers i col., 1991; Gabriel i col., 1999). L'activació reactiva de la glia és una de les respostes del sistema nerviós central front una lesió. Diferents autors han trobat que amb l'administració de l'MPTP en ratolins i gats s'indueix la reactivitat microglial (Kurkowska-Jastrezbscka y col., 1999) i astroglial (O'Callaghan y col., 1988; 1990a; 1992). Estudis previs han descrit que la degeneració

axonal pot induir activació microglial en zones llunyanes al lloc de la lesió (Gehrman i col., 1991; Fagan i Gage, 1994). Les cèl·lules microglials ramificades que normalment es troben presents en el cervell expressen el receptor del complement de tipus 3 CR3. Els resultats obtinguts per tècniques immunohistoquímiques en el present treball ha permès observar que la immunoreactivitat per aquesta proteïna mitjançant l'anticòs MRC OX-42 incrementava en el nucli estriat dies després de la lesió. També s'observaren canvis en la immunoreactivitat per l'OX-6, anticòs que reconeix el complex major d'histocompatibilitat de classe I (MHC I), i que s'utilitza com a marcador de la microglia reactiva i fagocítica. Quan hi ha una lesió s'activa la microglia en repòs i aquesta pot adoptar una forma hiperamificada i/o espinosa. En el cas de que es produeixi la mort neuronal pot adquirir una forma ameboide amb una morfologia similar a un macròfag (Streit i col., 1999). En el nostre model la microglia que s'observava en el nucli estriat ipsilateral a la lesió mostrava una morfologia reactiva espinosa i amb més ramificacions, sense arribar però, a la morfologia fagocítica. Aquests diferents canvis morfològics de les cèl·lules microglials, que acompanyen els diferents estadis de l'activitat microglial en funció del grau de lesió, han estat ja descrits (Wilson i Molliver, 1994; Kreutzberg, 1996; Streit i col, 1999). Junt a la reactivitat microglial, els astròcits també es tornaren reactius tal i com es va poder observar per l'increment d'expressió de la GFAP en el nucli estriat ipsilateral a la lesió. Els astròcits activats expressaven la proteïna de l'estrés Hsp27, que no s'expressa normalment en condicions fisiològiques normals però que és molt induïble després de situacions d'estrés (Kato i col., 1995; Plumier i col., 1996).

La reacció glial secundària observada en el nucli estriat depenia de la dosi de l'MPP^+ injectada a la substància negra, tal i com es va poder observar per la densitat dels receptors benzodiacepínics perifèrics, com per la intensitat i extensió de la immunoreactivitat microglial i astroglial. Sorprenentment aquesta dependència de la dosi en la resposta no es va observar en la pèrdua de innervació dopaminèrgica amb el GBR-12935 tritiat. De fet el paràmetre de unió estudiat, indicador de la captació de dopamina, a dosis baixes d'MPP^+ ja va arribar al seu nivell més baix, mentre que la reactivitat glial era més gran al incrementar la dosi de la neurotoxina. Així doncs, podria

ser que la funció dopaminèrgica es perdés abans que s'alliberés qualsevol senyal capaç de implicar la reactivitat glial.

Els factors que poden desencadenar una activació microglial encara són desconeguts. Estudis previs desmostraren que les cèl·lules microglials proliferen en el cervell en resposta a una citocina circulant, l'interferó γ (Grau i col., 1997), i que l'entrada de substància des del torrent sanguini en el teixit podien activar l'astroglia i la microglia (Giulan i col., 1988). De tota manera la reacció glial que s'observava després de la injecció intraperitoneal de l'MPTP no semblava que fos mediada per productes derivats de la sang ja que la barrera hematoencefàlica no estava alterada (O'Callaghan y col., 1990, 1992; Kukowska-Jastrzebscka y col., 1999). Així mateix els nostres resultats de marcatge per albúmina van ser negatius, marcatge que s'utilitza per detectar trencament de la barrera hematoencefàlica, ja que en condicions fisiològiques la entrada de l'albúmina des de la sang al cervell és restringida (Nordborg i col., 1994; Reynolds i Morton, 1998). Per tant en el nostre model no hi havia alteració de la barrera hematoencefàlica en el nucli estriat per la injecció de l'MPP^+ en la substància negra de rata, i la reactivitat glial present no podia ser deguda a l'entrada de cèl·lules procedents del reg sanguini.

Un dany sever en el cervell que comporta mort neuronal indueix una activació forta de la microglia rodona/macrofags i també dels astròcits, que adopten una forma més gran. Tot i això, en el nostre model no es van detectar signes de dany en neurones estriatals en els talls tenyits per hematoxilina i eosina o per la tinció de cresyl violeta. Tampoc s'observà inducció de la proteïna de l'estres Hsp72 en el nucli estriat, paràmetre ampliament usat com a marcador de dany neuronal. Els marcadors de degeneració i anàlisis estereològics de densitat neuronal en el nucli estriat ipsilateral no van mostrar diferències respecte els controls. A banda de no observar-se mort necròtica pels diferents mètodes emprats, tampoc vàrem observar mort apopòtica, com demostra el fet de que no hi ha fragmentació de l'ADN a cap dels dies posteriors a la lesió. Per tant aquestes dades ens suggereixen que la reactivació glial en el nucli estriat és produït per dany als terminals de les neurones dopaminèrgiques de la substància negra compacta, més que per la pèrdua de neurones estriatals. Ja s'havia suggerit anteriorment, que a part de la mort local de neurones, també existeixen altres senyals que poden induir gliosis

(Jorgensen y col., 1993). De fet, l'expressió de la interleucina-6 en neurones de ratolins transgènics produeix astrocitosi reactiva i incrementa el nombre de cèl·lules microglials pero no produeix dany neuronal (Fattori y col., 1995).

Quan existeix mort neuronal en el nucli estriat per una lesió s'observa un patró específic de distribució de les cèl·lules glials, mentre que la microglia i els macròfags es concentren en el centre de l'àrea necròtica, els astròcits es distribueixen al voltant, en el marges d'aquesta zona, creant una barrera entre l'àrea lesionada i la no lesionada (Planas i col., 1998; Stoll i col., 1998). En el nostre model no es va trobar aquesta distribució espaial en la lesió per MPP^+, i la microglia espinosa o ramificada colocalitzava amb els astròcits en les mateixes àrees estriatals. Rio i col., (1995) han descrit que la microglia fagocítica ameboide podia alliberar alguns factors negatius pels astròcits. La distribució espaial trobada per la glia indicaria que la microglia reactiva, que encara no ha adquirit forma ameboide, no té perquè exercir efectes negatius sobre els astròcits, ja que aquests es mantenen propers a la microglia en aquest model.

El paper d'aquesta reactivitat glial és encara un punt controvers. Sembla ser que la microglia podria comportar-se com neuroprotectora o neurotòxica en funció dels senyals rebuts del seu entorn (Zhang i Federoff, 1996; Streit i col., 1999). Mentre alguns treballs descriuen la microglia com alliberadora de mediadors tòxics (Wood, 1995, Rogore i Tsirka, 1998), altres indiquen una secreció de factors neurotròfics associant la microglia a mecanismes de neuroprotecció. La microglia activada i els macròfags expressen factors neurotròfics i indueixen la regeneració dopaminèrgica en el nucli estriat lesionat (Batchelor y col., 1999). A més els astròcits podrien contribuir a la regeneració mitjançant l'alliberació de factors tròfics (Lindsay, 1979; Banker, 1980).

Totes aquestes dades ens podrien indicar que en el nostre model la reacció glial i astroglial observada en el nucli estriat pretendrien promoure la regeneració de les fibres dopaminèrgiques que s'originen en la substància negra *pars compacta* i que han estat lesionades per l'MPP^+.

Regulació de la resposta autocrina/paracrina de les neurones corticals induïda per la lesió excitotòxica del nucli estriat.

En el següent treball, s'utilitzà el model de la malaltia de Huntington per l'excitotoxicitat en el nucli nucli estriat. La injecció d'aminoàcids excitadors ha sigut objecte d'estudi degut a la implicació d'un procés d'excitotoxicitat en malalties neurodegeneratives. A més s'ha postulat que alteracions en el nivells de neurotrofines podrien estar implicats en la fisiopatologia d'aquestes malalties (Connor i Dragunow, 1998). Treballs anteriors havien descrit la regulació de les neurotrofines i dels seus receptors en el nucli nucli estriat després d'una lesió excitotòxica en aquest nucli (Canals i col., 1998; 1999). Com que les dependències tròfiques en el sistema nerviós adult, es poden veure afectades per varis tipus de lesions, la pèrdua del suport diana es podria veure compensat per un increment autocrí/paracrí dels factors tròfics (Giehl i col., 1998; Schütte i col., 2000). L'objectiu a estudiar era quina repercussió tenia l'excitotoxicitat en el nucli nucli estriat sobre la regulació de les neurotrofines i els seus receptors en una àrea que està innervant el nucli nucli estriat, com és l'escorça cerebral.

Els resultats obtinguts per la tècnica de riboprotecció mostraren que l'expressió del BDNF i de l'NT-3 era modificada en l'escorça cerebral per les injeccions intranucli estriatals de l'àcid quinolínic, agonista del receptor NMDA i per l'àcid kaínic, agonista del receptor no-NMDA, mentre que la injecció de l'AMPA (no NMDA) i l'ACPD (metabotròpic) no provocaven canvis en els seus nivells. L'excitotoxicitat induïda per l'àcid kaínic va incrementar el nivells d'ARNm del BDNF i de l'NT-3, encara que el patrons d'expressió foren diferents i els canvis es localitzaren en diferents zones de l'escorça cerebral, com es va poder detectar per la tècnica de la hibridació *in situ*. Per altra banda la regulació deguda a la injecció de l'àcid quinolínic en el nucli nucli estriat va ser oposada ja que l'expressió del BDNF va augmentar mentre que la de l'NT-3 va disminuir unes hores després de la lesió. En altres àrees cerebrals s'han descrit també canvis diferencials de la regulació d'aquestes dues neurotrofines (Rocamora i col., 1994; Canals i col., 1998). Ja que s'ha observat que el BDNF i l'NT-3 presenten efectes oposats sobre les arboritzacions dendrítiques corticales (McAllister i col., 1997). Aquests canvis d'expressió del BDNF i de l'NT-3 ens podrien indicar la importància de cada una d'elles en àrees corticals específiques, com també s'ha descrit prèviament en

diferents processos d'excitotoxicitat (Rocamora i col., 1994; Checa i col., 2000). Aquests resultats ens indiquen que depenent de la població afectada en el nucli estriat s'indueix una regulació de les neurotrofines específica i combinada.

En aquest treball també s'observà una regulació dels receptors per aquestes neurotrofines, el trkB i el trkC. La lesió del nucli nucli estriat va incrementar els nivells del receptor trkB de forma complerta i del truncat, resultats obtinguts per la tècnica de la protecció per la RNAse. El receptor trkB de forma complerta i una de les dues isoformes del receptor truncat, el T2 (Middlemas i col., 1997), s'expressen principalment en neurones (Frisén i col., 1993; Armani i col., 1995; Biffo i col. 1995; Alderson i col., 2000). La implicació del receptor truncat és encara molt polèmica, s'ha descrit *in vitro* com a regulador negatiu del receptor de cadena complerta (Bifo i col., 1995; Eide i col., 1996; Fryer i col., 1997). Aquesta funció s'ha atribuït a les cèl·lules no-neuronals, que expressen la isoforma T1 del receptor truncat (Middlemas i col., 1991; Frisén i col., 1993). El receptor truncat pot induir la seva pròpia senyalització independentment del receptor de cadena llarga (Baxer i col., 1997). Mitjançant la tècnica de la hibridació *in situ* s'utlitzaren dues sondes per evaluar la localització de l'increment dels nivells de l'ARNm, mentre que una sonda ens permetia localitzar l'increment del receptor trk B en l'escorça sense diferenciar la forma truncada i la complerta l'altra ens localitzava l'increment del receptor de forma complerta, ja que s'uneix al domini tirosina quinasa. Es van obtenir diferentes patrons d'expressió amb les dues sondes, mentre que es va detectar que la isoforma que conté el domini catalític s'incrementava principalment en les capes del neocortex II-III, V i VI, la sonda que marcava totes les isoformes del receptor incrementava el seu marcatge en totes les capes excepte en la capa molecular. Amb aquests resultats podem dir que en la capa IV sols es localitza un increment del receptor truncat trkB, sense poder descartar l'augment d'aquest en les altres capes. Aquest increment observat en la capa IV suggereix una acció tròfica directa d'aquesta isoforma. Yacoubian i Lo (2000) han descrit recentment una regulació diferent del creixement dendrític induït pel receptor truncat i pel receptor de cadena complerta, i que el diferent patró d'expressió de les dues formes del receptor en les capes corticals podria estar relacionada amb requeriments específics de cada població neuronal.

El receptor trkC, el receptor per l'NT-3, mostra una regulació específica en funció del receptor per glutamat estimulat, i sols la injecció intraestriatal d'AMPA o ACPD incrementen la seva expressió. Aquests agonistas no modificaven els nivells de NT-3, mentre si que ho feien el altres agonistes del receptor pel glutamat, el que ens indicaria que les neurones corticals poden modificar les seves dependències tròfiques ja sigui canviant el nivells de l'NT-3 o l'expressió del seu receptor, trkC. La diferent regulació del trkB i del trkC estan en concordança amb el resultats de altres treballs on s'indueixen convulsions (Barbany i Persson, 1993; Merlio i col., 1993).

Els efectes funcionals descrits dels canvis de neurotrofines induïts per les lesions serien de protecció contra el dany neuronal i l'estimulació de la reorganització sinàptica (Lindvall i col., 1994). En particular, s'ha proposat que canvis en el BDNF cortical podrien jugar una paper en la supervivència de neurones corticals i nucli estriatals (Altar i DiStefano, 1998; Kokaia i col., 1998; Canals i col., 2001). De totes formes, la regulació específica dels receptors trk en l'escorça recolzarien unes acciones locals tròfiques. A més, l'increment de neurotrofines i receptors trk pot constituir una resposta autocrina/paracrina de les neurones corticals ja que el BDNF i l'NT-3 protegeixen aquestes cèl·lules de varis tipus de lesió (Kume i col., 1997).

Variacions en l'expressió de les neurotrofines i els seus receptors pot ser interpretat com canvis plàstics i refinament de l'eficàcia de la transmissió sinàptica (per revisió: McAllister i col., 1999). Els nostres resultats mostren una regulació independent de les neurotrofines i els seus receptors després de la lesió en el nucli nucli estriat. A més, no hem observat cap correlació entre el grau de lesió i la regulació dels receptors trk en l'escorça cerebral. Una altra possible explicació és la regulació depenent de l'activitat dels factors neurotròfics que modulen una adaptació plàstica en l'escorça cerebral. El circuit cortico-ganglis basals-cortical és essencial en la selecció i execució dels moviments desitjats mitjançant una seqüència de vies inhibidores i excitadores. La injecció d'agonistes dels receptors del glutamat podrien modificar aquesta red complexa (Parent i Hazrati, 1995). A més, el BDNF i l'NT-3 podrien ser capaces de mediar les accions dependents de l'activitat entre les neurones corticals (Bonhoeffer, 1996; Marty i col., 1997; McAllister i col., 1999). Per tant, canvis d'expressió observats en els nostres

resultats podrien participar en la modulació de la transmissió sinàptica en el circuit cortico-ganglis basals-cortical.

Regulació del BDNF endògen en la substància negra *pars compacta* després de la lesió excitotòxica en l'estriat.

Després d'observar la regulació del BDNF en l'escorça cerebral vàrem continuar l'estudi en la substancia negra. Per aquest treball s'injectà àcid kaínic a l'estriat. Entre les 4 i les 24 hores deprés de la lesió, per la tècnica de la riboprotecció, es detectà un increment del BDNF. Aquest increment es localitzava en la substància negra compacta, tal i com es podia veure per la tècnica de la hibridació *in situ*.

S'ha descrit recentment que la lesió de la via nigroestriada per la 6-hidroxidopamina produïa un increment de l'expressió del BDNF en la substancia negra *pars reticulata* (Aliaga i col., 2000). Aquest increment d'expressió de les neurones no-dopaminègiques podria tenir un efecte paracrí sobre les neurones dopaminèrgiques lesionades de la substància negra *pars compacta*. En l'axotomia corticospinal també s'ha mostrat un increment d'aquesta neurotrofina amb el fi de protegir les neurones corticals (Giehl i col., 1998). En el nostre model s'observà també, que les neurones dopaminèrgiques no estan afectades per la manca de la diana, el nucli estriat lesionat per l'excitotoxicitat, el que ens mostra l'efecte tròfic autocrí o paracrí del BDNF per aquestes cèl·lules dopaminèrgiques. Aquesta neurotrofina té un paper important sobre les neurones dopaminèrgiques de la substancia negra com han descrit molts autors, la injecció intranigral de BDNF prevé la degeneració transneuronal de les neurones nigrals per les lesions excitotòxiques estriatals-pallidals (Volpe i col., 1998).

El mecanisme neuroprotector del BDNF encara es desconeix, però el fet que el BDNF incrementi les proteïnes queladores de calci en cultius (Ip i col., 1993), podria actuar tamponant l'increment toxic de calci intracel·lular. A més, l'excitotoxicitat també produeix estrés oxidatiu, que el BDNF podria reduir incrementant l'enzim glutatió reductasa com s'ha observat en cultius de neurones nigrals (Spina i col., 1992).

Les neurotrofines prevenen la pèrdua de neurones estriatals després de la lesió excitotòxica per quinolínic, model de la malaltia de Huntington.

La injecció de l'àcid quinolínic en el nucli nucli estriat reprodueix les característiques neuroquímiques, histològiques i els canvis comportamentals de la malaltia de Huntington (Beal i col., 1986; Difiglia, 1990) per el que se l'ha descrit com un bon model d'estudi per aquesta malaltia. Les neurotrofines s'han proposat com a bones candidates per a la teràpia d'alteracions neurodegeneratives degut a que promouen la supervivència de diferents poblacions neuronales (Connor i Dragunow, 1998). En aquest treball es va estudiar el possible efecte protector del BDNF, de l'NT-3 i de l'NT-4/5 sobre les projeccions nucli estriatals en el model de l'àcid quinolínic. Per dur a terme aquest treball es van implantar línies cel·lulars secretores de les diferents neurotrofines en el nucli nucli estriat, posteriorment lesionat amb l'agonista del receptor NMDA.

El número de cèl·lules estriatals TUNEL positives, marcador de mort neuronal, va disminuir en presència de cada una de les neurotrofines, essent el BDNF el factor més potent. Aquesta potència es mantingué a l'estudiar les diferents poblacions de projecció, estudi que es dugué a terme mitjançant la tècnica de la hibridació *in situ*, i en la que s'utilitzaren com a marcadors l'enzim glutàmic àcid decarboxilasa (GAD), la preproencefalina (PPE), la preprotaquiquinina (PPTA) o la dinorfina (DYN). A diferencia del BDNF, que protegia totes les poblacions de projecció nucli estriatals, l'NT-3 i l'NT4/5 no prevenien la pèrdua de neurones dinorfina i eren menys eficaces en la protecció de les neurones GAD positives. Aquesta acció de protecció es podria explicar com una acció directa sobre els seus receptores, degut a l'expressió dels receptors trkB i trkC en les neurones de projecció estriatals. En concordància amb aquests resultats existeixen treballs *in vitro* i *in vivo* que han descrit el BDNF, l'NT-3 i l'NT-4/5 com promotores de la supervivència de les neurones GABAèrgiques nucli estriatals (Widmer i Hefti, 1994; Ventimiglia i col., 1998) i que les neurotrofines protegeixen front a diferents tipus de lesió (Nakao i col., 1995a,b). Però altres estudis no van trobar cap efecte del BDNF (Frim i col., 1993; Anderson i col., 1996) i de l'NT-3 (Anderson i col, 1996). Les diferències metodològiques per l'estudi de la mort neuronal, així com el grau de lesió i/o les altes dosis de neurotrofines utilitzades podrien explicar l'absència d'efectes. De fet exposicions llargues d'altes dosis de BDNF indueixen una

desensibilització del receptor trkB (Carter i col., 1995; Frank i col., 1996, Knüssel i col., 1996), suggerint que el tractament amb altes dosis de neurotrofines podria reduir la resposta dels receptors. A més dosis baixes de BDNF in vivo no han disminuït la resposta del BDNF (Knüsel i col., 1996), han induït l'expressió del trkB (Ferrer i col., 1998) i s'ha observat una protecció de les projeccions estriatals front la lesió de l'àcid quinolínic (Martínez-Serrano i Björklund, 1996, Bemelmans i col., 1999; present estudi). Cal destacar que s'han trobat diferències en quan a la protecció de la supervivència (present treball) i del fenotip (Pérez-Navarro i col., 1999a) de les neurones de projecció el que correspondria amb el concepte de les diferents vies intracel·lulars implicades en la inducció de la supervivència i/o diferenciació que tenen lloc després de la unió de la neurotrofina amb el seu receptor (para revisió Segal i Greenberg, 1996; Springer i Kitzman, 1998).

Resultats anteriors van mostrar que les neurotrofines podien presentar efectes sobre el fenotip de les neurones de projecció tant si eren alliberades en la substància negra (Arenas i col., 1996) com en el nucli nucli estriat (Pérez-Navarro i col., 1999a). S'han descrit que el BDNF era transportat anteròg radament des de l'escorça cerebral (Altar i col., 1997), i es coneix l'expressió del BDNF en òrgans diana del nucli nucli estriat, la substància negra i el *globus pallidus* (Miranda i col., Seroogy i Gall, 1993; Timmusk i col., 1993). En aquest treball es mostra que les neurotrofines poden prevenir la degeneració de les projeccions nucli estriatals actuant d'una forma autocrina/paracrina. Aquesta forma d'acció estaria en concordància amb la presència dels axons recurrents col·laterals de les projeccions en el nucli estriat.

Els mecanismes inductors de la neurodegeneració present en la malaltia de Huntington són desconeguts, encara que s'ha proposat que l'activitat caspasa juntament amb l'excitotoxicitat, l'estrés oxidatiu i alteracions metabòliques podrien ser possibles mecanismes (Golberg i col., 1996; Pétersén i col., 1999). Ona i els seus col·laboradors (1999) van mostrar un enlentiment de la progressió de la malaltia inhibint la caspasa-1. A més, alguns treballs han descrit que els factors neurotròfics podrien exercir la seva acció neuroprotectora mitjançant la inhibició de l'activitat caspasa *in vitro* (Tamatani i col., 1998) i *in vivo* (Han i col., 2000). Les neurotrofines podrien actuar mitjançant mecanismes que tenen lloc durant el desenvolupament. S'ha vist que ratolins mutants pel

trkB mostraven més mort cel·lular apoptòtica durant el desenvolupament (Alcantara i col., 1998). Totes aquestes dades proposen un paper important als factors tròfics durant el desenvolupament i en la regeneració de neurones estriatals, pel que l'administració de baixes dosis de BDNF podria ser una teràpia important en les malalties degeneratives que afecten les neurones estriatasl de projecció.

CONCLUSIONS

1.-La injecció de l'MPP^+ en la substància negra només afecta els terminals dopaminèrgics sense induir mort cel·lular en el nucli estriat, ni alterar la barrera hematoencefàlica.

2.-La degeneració dels terminals dopaminèrgics indueix una activació de les cèl·lules glials residents que podria estar implicada en fenòmens de plasticitat.

3.-El BDNF i la NT-3 i els seus receptors tenen una diferent regulació en l'escorça cerebral després de la lesió del nucli estriat. Aquesta regulació és una resposta autocrina/paracrina de les neurones corticals per protegir-se de la pèrdua del suport tròfic del nucli estriat

4.-La independent regulació cortical dels receptors trks amb regulació del BDNF i de la NT-3 representa un mecanisme tròfic per la proteció de les diferents poblacions neuronals de l'escorça cerebral.

5.-La injecció intraestriatal d'àcid kainic produeix degeneració transneuronal en la substancia negra *pars reticulata*, mentre que les neurones dopaminèrgiques de la *pars compacta* no es veuen afecades per la manca del nucli diana.

6.-Els nivells d'ARNm del BDNF incrementen les hores posteriors a la lesió excitotòxica del nucli estriat, aquest increment localitzat en la substància negra *pars compacta*, es una resposta tròfica autocrina/paracrina que protegeix les neurones dopaminèrgiques de la degeneració transneuronal.

7.-Les neurotrofines BDNF, NT-3 i NT-4/5 protegeixen de la mort cel·lular estriatal causada per la injecció de l'àcid quinolínic, el que indicaria que el suport tròfic ve determinat per una complexa interacció entre les diferents neurotrofines.

8.-El BDNF és el factor més potent en promoure la supervivència de les neurones de projecció en un model experimental de la malaltia de Huntington. Per això aquesta neurotrofina podria tenir una paper important en el tractament de les malalties degeneratives on les neurones de projecció estan afectades.

BIBLIOGRAFIA

Abiru, Y., Nishio, C. & Hatanaka, H. (1996) The survival of striatal cholinergic neurons cultured from postnatal 2-week-old rats is promoted by neurotrophins. *Brain Res Dev Brain Res*, **91**, 260-267.

Akerud, P., Alberch, J., Eketjall, S., Wagner, J. & Arenas, E. (1999) Differential effects of glial cell line-derived neurotrophic factor and neurturin on developing and adult substantia nigra dopaminergic neurons. *J Neurochem*, **73**, 70-78.

Akiyama, H., Tooyama, I., Kondo, H., Ikeda, K., Kimura, H., McGeer, E.G. & McGeer, P.L. (1994) Early response of brain resident microglia to kainic acid-induced hippocampal lesions. *Brain Res*, **635**, 257-268.

Albin, R.L., Young, A.B. & Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci*, **12**, 366-375.

Alcantara, S., Frisen, J., del Rio, J.A., Soriano, E., Barbacid, M. & Silos-Santiago, I. (1997) TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death. *J Neurosci*, **17**, 3623-3633.

Alexi, T. & Hefti, F. (1996) Neurotrophin-4/5 selectively protects nigral calbindin-containing neurons in rats with medial forebrain bundle transections. *Neuroscience*, **72**, 911-921.

Alexi, T., Venero, J.L. & Hefti, F. (1997) Protective effects of neurotrophin-4/5 and transforming growth factor-alpha on striatal neuronal phenotypic degeneration after excitotoxic lesioning with quinolinic acid. *Neuroscience*, **78**, 73-86.

Alexi, T., Hughes, P.E., Knusel, B. & Tobin, A.J. (1998) Metabolic compromise with systemic 3-nitropropionic acid produces striatal apoptosis in Sprague-Dawley rats but not in BALB/c ByJ mice. *Exp Neurol*, **153**, 74-93.

Alexi, T., Hughes, P.E., Faull, R.L. & Williams, C.E. (1998) 3-Nitropropionic acid's lethal triplet: cooperative pathways of neurodegeneration. *Neuroreport*, **9**, R57-R64.

Altar, C.A., Boylan, C.B., Fritsche, M., Jackson, C., Hyman, C. & Lindsay, R.M. (1994) The neurotrophins NT-4/5 and BDNF augment serotonin, dopamine, and GABAergic systems during behaviorally effective infusions to the substantia nigra. *Exp Neurol*, **130**, 31-40.

Altar, C.A., Siuciak, J.A., Wright, P., Ip, N.Y., Lindsay, R.M. & Wiegand, S.J. (1994) In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [125I]BDNF, [125I]NT-4/5 and [125I]NT-3. *Eur J Neurosci*, **6**, 1389-1405.

Altar, C.A., Cai, N., Bliven, T., Juhasz, M., Conner, J.M., Acheson, A.L., Lindsay, R.M. & Wiegand, S.J. (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature*, **389**, 856-860.

Anderson, C.M. & Swanson, R.A. (2000) Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia*, **32**, 1-14.

Anderson, K.D., Panayotatos, N., Corcoran, T.L., Lindsay, R.M. & Wiegand, S.J. (1996) Ciliary neurotrophic factor protects striatal output neurons in an animal model of Huntington disease. *Proc Natl Acad Sci U S A*, **93**, 7346-7351.

Angeletti, P. & Vigneti, E. (1971) Assay of nerve growth factor (NGF) in subcellular fractions of peripheral tissues by micro complement fixation. *Brain Res*, **33**, 601-604.

Angeletti, P.U. & Levi-Montalcini, R. (1971) Growth regulation of the sympathetic nervous system: immunosympathectomy and chemical sympathectomy. *Rev Eur Etud Clin Biol*, **16**, 866-874.

Angeletti, P.U., Levi-Montalcini, R. & Caramia, F. (1971) Ultrastructural changes in sympathetic neurons of newborn and adult mice treated with nerve growth factor. *J Ultrastruct Res*, **36**, 24-36.

Angeletti, P.U., Levi-Montalcini, R. & Caramia, F. (1971) Analysis of the effects of the antiserum to the nerve growth factor in adult mice. *Brain Res*, **27**, 343-355.

Angeletti, P.U. (1972) Biological properties of the nerve growth factor. *Adv Exp Med Biol,* **32**, 83-90.

Angeletti, R.H. & Bradshaw, R.A. (1971) Nerve growth factor from mouse submaxillary gland: amino acid sequence. *Proc Natl Acad Sci U S A,* **68**, 2417-2420.

Angeletti, R.H. (1971) Immunological relatedness of nerve growth factors. *Brain Res,* **25**, 424-427.

Angeletti, R.H., Frazier, W.A. & Bradshaw, R.A. (1972) Structural studies of 2.5 S mouse submaxillary gland nerve growth factor. *Adv Exp Med Biol,* **32**, 99-106.

Anholt, R.R., Murphy, K.M., Mack, G.E. & Snyder, S.H. (1984) Peripheral-type benzodiazepine receptors in the central nervous system: localization to olfactory nerves. *J Neurosci,* **4**, 593-603.

Araujo, D.M. & Cotman, C.W. (1992) Basic FGF in astroglial, microglial, and neuronal cultures: characterization of binding sites and modulation of release by lymphokines and trophic factors. *J Neurosci,* **12**, 1668-1678.

Ardelt, A.A., Flaris, N.A. & Roth, K.A. (1994) Neurotrophin-4 selectively promotes survival of striatal neurons in organotypic slice culture. *Brain Res,* **647**, 340-344.

Arenas, E., Akerud, P., Wong, V., Boylan, C., Persson, H., Lindsay, R.M. & Altar, C.A. (1996) Effects of BDNF and NT-4/5 on striatonigral neuropeptides or nigral GABA neurons in vivo. *Eur J Neurosci,* **8**, 1707-1717.

Ashizawa, T., Wong, L.J., Richards, C.S., Caskey, C.T. & Jankovic, J. (1994) CAG repeat size and clinical presentation in Huntington's disease. *Neurology,* **44**, 1137-1143.

Augood, S.J., Faull, R.L., Love, D.R. & Emson, P.C. (1996) Reduction in enkephalin and substance P messenger RNA in the striatum of early grade Huntington's disease: a detailed cellular in situ hybridization study. *Neuroscience,* **72**, 1023-1036.

Baker, S.J. & Reddy, E.P. (1998) Modulation of life and death by the TNF receptor superfamily. *Oncogene*, **17**, 3261-3270.

Banati, R.B. & Kreutzberg, G.W. (1993) Flow cytometry: measurement of proteolytic and cytotoxic activity of microglia. *Clin Neuropathol*, **12**, 285-288.

Banati, R.B., Gehrmann, J., Schubert, P. & Kreutzberg, G.W. (1993) Cytotoxicity of microglia. *Glia*, **7**, 111-118.

Banati, R.B., Gehrmann, J., Czech, C., Monning, U., Jones, L.L., Konig, G., Beyreuther, K. & Kreutzberg, G.W. (1993) Early and rapid de novo synthesis of Alzheimer beta A4-amyloid precursor protein (APP) in activated microglia. *Glia*, **9**, 199-210.

Bandmann, O., Marsden, C.D. & Wood, N.W. (1998) Genetic aspects of Parkinson's disease. *Mov Disord*, **13**, 203-211.

Banker, G.A. (1980) Trophic interactions between astroglial cells and hippocampal neurons in culture. *Science*, **209**, 809-810.

Barbacid, M. (1994) The Trk family of neurotrophin receptors. *J Neurobiol*, **25**, 1386-1403.

Barbacid, M. (1995) Structural and functional properties of the TRK family of neurotrophin receptors. *Ann N Y Acad Sci*, **766**, 442-458.

Barbacid, M. (1995) Neurotrophic factors and their receptors. *Curr Opin Cell Biol*, **7**, 148-155.

Barde, Y.A., Edgar, D. & Thoenen, H. (1982) Purification of a new neurotrophic factor from mammalian brain. *EMBO J*, **1**, 549-553.

Batchelor, P.E., Liberatore, G.T., Wong, J.Y., Porritt, M.J., Frerichs, F., Donnan, G.A. & Howells, D.W. (1999) Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor. *J Neurosci*, **19**, 1708-1716.

Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J. & Martin, J.B. (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. *Nature*, **321**, 168-171.

Beal, M.F., Kowall, N.W., Swartz, K.J., Ferrante, R.J. & Martin, J.B. (1989) Differential sparing of somatostatin-neuropeptide Y and cholinergic neurons following striatal excitotoxin lesions. *Synapse*, **3**, 38-47.

Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R. & Hyman, B.T. (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *J Neurosci*, **13**, 4181-4192.

Beck, K.D., Valverde, J., Alexi, T., Poulsen, K., Moffat, B., Vandlen, R.A., Rosenthal, A. & Hefti, F. (1995) Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. *Nature*, **373**, 339-341.

Beckstead, R.M., Domesick, V.B. & Nauta, W.J. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res*, **175**, 191-217.

Beckstead, R.M. & Kersey, K.S. (1985) Immunohistochemical demonstration of differential substance P-, met-enkephalin-, and glutamic-acid-decarboxylase-containing cell body and axon distributions in the corpus striatum of the cat. *J Comp Neurol*, **232**, 481-498.

Bemelmans, A.P., Horellou, P., Pradier, L., Brunet, I., Colin, P. & Mallet, J. (1999) Brain-derived neurotrophic factor-mediated protection of striatal neurons in an excitotoxic rat model of Huntington's disease, as demonstrated by adenoviral gene transfer. *Hum Gene Ther*, **10**, 2987-2997.

Benavides, J., Fage, D., Carter, C. & Scatton, B. (1987) Peripheral type benzodiazepine binding sites are a sensitive indirect index of neuronal damage. *Brain Res*, **421**, 167-172.

Benavides, J., Capdeville, C., Dauphin, F., Dubois, A., Duverger, D., Fage, D., Gotti, B., MacKenzie, E.T. & Scatton, B. (1990) The quantification of brain lesions with an omega 3 site ligand: a critical analysis of animal models of cerebral ischaemia and neurodegeneration. *Brain Res*, **522**, 275-289.

Benavides, J., Bourdiol, F., Dubois, A. & Scatton, B. (1991) Regional pattern of increased omega 3 (peripheral type benzodiazepine) binding site densities in the rat brain induced by systemic injection of kainic acid. *Neurosci Lett*, **125**, 219-222.

Bender, A.S. & Hertz, L. (1987) Pharmacological characteristics of diazepam receptors in neurons and astrocytes in primary cultures. *J Neurosci Res*, **18**, 366-372.

Bennett, B.D. & Bolam, J.P. (1993) Characterization of calretinin-immunoreactive structures in the striatum of the rat. *Brain Res*, **609**, 137-148.

Bergles, D.E. & Jahr, C.E. (1998) Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. *J Neurosci*, **18**, 7709-7716.

Berninger, B. & Poo, M. (1996) Fast actions of neurotrophic factors. *Curr Opin Neurobiol*, **6**, 324-330.

Biffo, S., Offenhauser, N., Carter, B.D. & Barde, Y.A. (1995) Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. *Development*, **121**, 2461-2470.

Blesch, A., Grill, R.J. & Tuszynski, M.H. (1998) Neurotrophin gene therapy in CNS models of trauma and degeneration. *Prog Brain Res*, **117**, 473-484.

Bolam, J.P., Wainer, B.H. & Smith, A.D. (1984) Characterization of cholinergic neurons in the rat neostriatum. A combination of choline acetyltransferase immunocytochemistry, Golgi-impregnation and electron microscopy. *Neuroscience*, **12**, 711-718.

Bolam, J.P., Ingham, C.A. & Smith, A.D. (1984) The section-Golgi-impregnation procedure--3. Combination of Golgi-impregnation with enzyme histochemistry and

electron microscopy to characterize acetylcholinesterase-containing neurons in the rat neostriatum. *Neuroscience*, **12**, 687-709.

Bolam, J.P., Hanley, J.J., Booth, P.A. & Bevan, M.D. (2000) Synaptic organisation of the basal ganglia. *J Anat*, **196 (Pt 4)**, 527-542.

Bonhoeffer, T. (1996) Neurotrophins and activity-dependent development of the neocortex. *Curr Opin Neurobiol*, **6**, 119-126.

Borlongan, C.V., Nishino, H. & Sanberg, P.R. (1997) Systemic, but not intraparenchymal, administration of 3-nitropropionic acid mimics the neuropathology of Huntington's disease: a speculative explanation. *Neurosci Res*, **28**, 185-189.

Borlongan, C.V., Koutouzis, T.K. & Sanberg, P.R. (1997) 3-Nitropropionic acid animal model and Huntington's disease. *Neurosci Biobehav Rev*, **21**, 289-293.

Bothwell, M. (1995) Functional interactions of neurotrophins and neurotrophin receptors. *Annu Rev Neurosci*, **18**, 223-253.

Boyes, B.E., Kim, S.U., Lee, V. & Sung, S.C. (1986) Immunohistochemical co-localization of S-100b and the glial fibrillary acidic protein in rat brain. *Neuroscience*, **17**, 857-865.

Bradbury, A.J., Costall, B., Jenner, P.G., Kelly, M.E., Marsden, C.D. & Naylor, R.J. (1986) MPP+ can disrupt the nigrostriatal dopamine system by acting in the terminal area. *Neuropharmacology*, **25**, 939-941.

Bradbury, A.J., Brossi, A., Costall, B., Domeney, A.M., Gessner, W. & Naylor, R.J. (1986) Biochemical changes caused by the infusion into the substantia nigra of the rat of MPTP and related compounds which antagonise dihydropteridine reductase. *Neuropharmacology*, **25**, 583-586.

Bradbury, A.J., Costall, B., Domeney, A.M., Jenner, P., Kelly, M.E., Marsden, C.D. & Naylor, R.J. (1986) 1-methyl-4-phenylpyridine is neurotoxic to the nigrostriatal dopamine pathway. *Nature*, **319**, 56-57.

Bradbury, A.J., Costall, B., Jenner, P.G., Kelly, M.E., Marsden, C.D. & Naylor, R.J. (1986) The effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal and limbic catecholamine neurones in white and black mice. Antagonism by monoamine oxidase inhibitors. *Neuropharmacology,* **25**, 897-904.

Brandt, J., Bylsma, F.W., Gross, R., Stine, O.C., Ranen, N. & Ross, C.A. (1996) Trinucleotide repeat length and clinical progression in Huntington's disease. *Neurology,* **46**, 527-531.

Brouillet, E., Hantraye, P., Ferrante, R.J., Dolan, R., Leroy-Willig, A., Kowall, N.W. & Beal, M.F. (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc Natl Acad Sci U S A,* **92**, 7105-7109.

Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M. & Kopin, I.J. (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci U S A,* **80**, 4546-4550.

Canals, J.M., Marco, S., Checa, N., Michels, A., Perez-Navarro, E., Arenas, E. & Alberch, J. (1998) Differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 after excitotoxicity in a rat model of Huntington's disease. *Neurobiol Dis,* **5**, 357-364.

Canals, J.M., Checa, N., Marco, S., Michels, A., Perez-Navarro, E. & Alberch, J. (1999) The neurotrophin receptors trkA, trkB and trkC are differentially regulated after excitotoxic lesion in rat striatum. *Brain Res Mol Brain Res,* **69**, 242-248.

Carmignoto, G. (2000) Reciprocal communication systems between astrocytes and neurones. *Prog Neurobiol,* **62**, 561-581.

Carter, B.D., Zirrgiebel, U. & Barde, Y.A. (1995) Differential regulation of p21ras activation in neurons by nerve growth factor and brain-derived neurotrophic factor. *J Biol Chem,* **270**, 21751-21757.

Carter, B.D. & Lewin, G.R. (1997) Neurotrophins live or let die: does p75NTR decide? *Neuron*, **18**, 187-190.

Castellani, V. & Bolz, J. (1999) Opposing roles for neurotrophin-3 in targeting and collateral formation of distinct sets of developing cortical neurons. *Development*, **126**, 3335-3345.

Celio, M.R. (1990) Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience*, **35**, 375-475.

Chanas-Sacre, G., Rogister, B., Moonen, G. & Leprince, P. (2000) Radial glia phenotype: origin, regulation, and transdifferentiation. *J Neurosci Res*, **61**, 357-363.

Chao, M.V. (1994) The p75 neurotrophin receptor. *J Neurobiol*, **25**, 1373-1385.

Checa, N., Canals, J.M. & Alberch, J. (2000) Developmental regulation of BDNF and NT-3 expression by quinolinic acid in the striatum and its main connections. *Exp Neurol*, **165**, 118-124.

Cheng, B., Goodman, Y., Begley, J.G. & Mattson, M.P. (1994) Neurotrophin-4/5 protects hippocampal and cortical neurons against energy deprivation- and excitatory amino acid-induced injury. *Brain Res*, **650**, 331-335.

Cheng, B. & Mattson, M.P. (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. *Brain Res*, **640**, 56-67.

Chesselet, M.F. & Graybiel, A.M. (1986) Striatal neurons expressing somatostatin-like immunoreactivity: evidence for a peptidergic interneuronal system in the cat. *Neuroscience*, **17**, 547-571.

Chesselet, M.F. & Robbins, E. (1989) Characterization of striatal neurons expressing high levels of glutamic acid decarboxylase messenger RNA. *Brain Res*, **492**, 237-244.

Chiba, K., Trevor, A. & Castagnoli, N.J. (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun,* **120**, 574-578.

Chiba, K., Trevor, A.J. & Castagnoli, N.J. (1985) Active uptake of MPP+, a metabolite of MPTP, by brain synaptosomes. *Biochem Biophys Res Commun,* **128**, 1228-1232.

Chiba, K., Peterson, L.A., Castagnoli, K.P., Trevor, A.J. & Castagnoli, N.J. (1985) Studies on the molecular mechanism of bioactivation of the selective nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Drug Metab Dispos,* **13**, 342-347.

Chiueh, C.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.

Chvatal, A. & Sykova, E. (2000) Glial influence on neuronal signaling. *Prog Brain Res,* **125**, 199-216.

Cicchetti, F., Beach, T.G. & Parent, A. (1998) Chemical phenotype of calretinin interneurons in the human striatum. *Synapse,* **30**, 284-297.

Cohen, G. & Heikkila, R.E. (1974) The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J Biol Chem,* **249**, 2447-2452.

Colton, C.A. & Gilbert, D.L. (1987) Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Lett,* **223**, 284-288.

Connor, B. & Dragunow, M. (1998) The role of neuronal growth factors in neurodegenerative disorders of the human brain. *Brain Res Brain Res Rev,* **27**, 1-39.

Conover, J.C., Erickson, J.T., Katz, D.M., Bianchi, L.M., Poueymirou, W.T., McClain, J., Pan, L., Helgren, M., Ip, N.Y. & Boland, P. (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. *Nature,* **375**, 235-238.

Cowan, R.L., Wilson, C.J., Emson, P.C. & Heizmann, C.W. (1990) Parvalbumin-containing GABAergic interneurons in the rat neostriatum. *J Comp Neurol,* **302**, 197-205.

Coyle, J.T. & Schwarcz, R. (1976) Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. *Nature,* **263**, 244-246.

Dahlstrom, A. & Fuxe, K. (1964) Localization of monoamines in the lower brain stem. *Experientia,* **20**, 398-399.

Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L. & Bates, G.P. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell,* **90**, 537-548.

Dawbarn, D., De Quidt, M.E. & Emson, P.C. (1985) Survival of basal ganglia neuropeptide Y-somatostatin neurones in Huntington's disease. *Brain Res,* **340**, 251-260.

Dechant, G., Rodriguez-Tebar, A. & Barde, Y.A. (1994) Neurotrophin receptors. *Prog Neurobiol,* **42**, 347-352.

DeFazio, R.A., Pong, K., Knusel, B. & Walsh, J.P. Neurotrophin-4/5 promotes dendritic outgrowth and calcium currents in cultured mesencephalic dopamine neurons. *Neuroscience 2000. ;99. (2.):297. -304.*, **99**, 297-304.

Deutch, A.Y., Goldstein, M. & Roth, R.H. (1986) The ascending projections of the dopaminergic neurons of the substantia nigra, zona reticulata: a combined retrograde tracer-immunohistochemical study. *Neurosci Lett,* **71**, 257-263.

Diemel, L.T., Brewster, W.J., Fernyhough, P. & Tomlinson, D.R. (1994) Expression of neuropeptides in experimental diabetes; effects of treatment with nerve growth factor or brain-derived neurotrophic factor. *Brain Res Mol Brain Res*, **21**, 171-175.

DiFiglia, M. & Aronin, N. (1982) Ultrastructural features of immunoreactive somatostatin neurons in the rat caudate nucleus. *J Neurosci*, **2**, 1267-1274.

DiFiglia, M. & Aronin, N. (1984) Quantitative electron microscopic study of immunoreactive somatostatin axons in the rat neostriatum. *Neurosci Lett*, **50**, 325-331.

DiFiglia, M. (1990) Excitotoxic injury of the neostriatum: a model for Huntington's disease. *Trends Neurosci*, **13**, 286-289.

Doble, A. (1995) Excitatory amino acid receptors and neurodegeneration. *Therapie*, **50**, 319-337.

Dragunow, M., Beilharz, E., Sirimanne, E., Lawlor, P., Williams, C., Bravo, R. & Gluckman, P. (1994) Immediate-early gene protein expression in neurons undergoing delayed death, but not necrosis, following hypoxic-ischaemic injury to the young rat brain. *Brain Res Mol Brain Res*, **25**, 19-33.

Dringen, R. & Hamprecht, B. (1998) Glutathione restoration as indicator for cellular metabolism of astroglial cells. *Dev Neurosci*, **20**, 401-407.

Dringen, R. (2000) Metabolism and functions of glutathione in brain. *Prog Neurobiol*, **62**, 649-671.

Dusart, I. & Schwab, M.E. (1994) Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur J Neurosci*, **6**, 712-724.

Eddleston, M. & Mucke, L. (1993) Molecular profile of reactive astrocytes--implications for their role in neurologic disease. *Neuroscience*, **54**, 15-36.

Eide, F.F., Vining, E.R., Eide, B.L., Zang, K., Wang, X.Y. & Reichardt, L.F. (1996) Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J Neurosci,* **16**, 3123-3129.

Enfors, S.O., Hellebust, H., Kohler, K., Strandberg, L. & Veide, A. (1990) Impact of genetic engineering on downstream processing of proteins produced in E. coli. *Adv Biochem Eng Biotechnol,* **43**, 31-42.

Eng, L.F., Lee, Y.L., Murphy, G.M. & Yu, A.C. (1995) A RT-PCR study of gene expression in a mechanical injury model. *Prog Brain Res,* **105**, 219-229.

Ernfors, P., Lee, K.F. & Jaenisch, R. (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature,* **368**, 147-150.

Fagan, A.M. & Gage, F.H. (1994) Mechanisms of sprouting in the adult central nervous system: cellular responses in areas of terminal degeneration and reinnervation in the rat hippocampus. *Neuroscience,* **58**, 705-725.

Fallon, J.H., Wang, C., Kim, Y., Canepa, N., Loughlin, S. & Seroogy, K. (1983) Dopamine- and cholecystokinin-containing neurons of the crossed mesostriatal projection. *Neurosci Lett,* **40**, 233-238.

Fallon, J.H. & Seroogy, K.B. (1985) The distribution and some connections of cholecystokinin neurons in the rat brain. *Ann N Y Acad Sci,* **448**, 121-132.

Farinas, I., Jones, K.R., Backus, C., Wang, X.Y. & Reichardt, L.F. (1994) Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. *Nature,* **369**, 658-661.

Faull, R.L. & Mehler, W.R. (1978) The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. *Neuroscience,* **3**, 989-1002.

Ferrante, R.J., Gutekunst, C.A., Persichetti, F., McNeil, S.M., Kowall, N.W., Gusella, J.F., MacDonald, M.E., Beal, M.F. & Hersch, S.M. (1997) Heterogeneous

topographic and cellular distribution of huntingtin expression in the normal human neostriatum. *J Neurosci,* **17**, 3052-3063.

Ferrer, I., Ballabriga, J., Marti, E., Perez, E., Alberch, J. & Arenas, E. (1998) BDNF up-regulates TrkB protein and prevents the death of CA1 neurons following transient forebrain ischemia. *Brain Pathol,* **8**, 253-261.

Folstein, S.E. (1991) The psychopathology of Huntington's disease. *Res Publ Assoc Res Nerv Ment Dis,* **69**, 181-191.

Folstein, S.E. (1991) The psychopathology of Huntington's disease. *Res Publ Assoc Res Nerv Ment Dis,* **69**, 181-191.

Frade, J.M. & Barde, Y.A. (1999) Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. *Development,* **126**, 683-690.

Fraher, J.P. (2000) The transitional zone and CNS regeneration. *J Anat,* **196 (Pt 1)**, 137-158.

Frank, L., Ventimiglia, R., Anderson, K., Lindsay, R.M. & Rudge, J.S. (1996) BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. *Eur J Neurosci,* **8**, 1220-1230.

Frim, D.M., Uhler, T.A., Galpern, W.R., Beal, M.F., Breakefield, X.O. & Isacson, O. (1994) Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. *Proc Natl Acad Sci U S A,* **91**, 5104-5108.

Frisen, J., Verge, V.M., Fried, K., Risling, M., Persson, H., Trotter, J., Hokfelt, T. & Lindholm, D. (1993) Characterization of glial trkB receptors: differential response to injury in the central and peripheral nervous systems. *Proc Natl Acad Sci U S A,* **90** , 4971-4975.

Gall, C. & Lauterborn, J. (1992) The dentate gyrus: a model system for studies of neurotrophin regulation. *Epilepsy Res Suppl*, **7:171-85.**, 171-185.

Galpern, W.R., Frim, D.M., Tatter, S.B., Altar, C.A., Beal, M.F. & Isacson, O. (1996) Cell-mediated delivery of brain-derived neurotrophic factor enhances dopamine levels in an MPP+ rat model of substantia nigra degeneration. *Cell Transplant*, **5**, 225-232.

Gehlert, D.R., Yamamura, H.I. & Wamsley, J.K. (1985) Autoradiographic localization of "peripheral-type" benzodiazepine binding sites in the rat brain, heart and kidney. *Naunyn Schmiedebergs Arch Pharmacol*, **328**, 454-460.

Gehrmann, J., Schoen, S.W. & Kreutzberg, G.W. (1991) Lesion of the rat entorhinal cortex leads to a rapid microglial reaction in the dentate gyrus. A light and electron microscopical study. *Acta Neuropathol (Berl)*, **82**, 442-455.

Gehrmann, J. (1995) Colony-stimulating factors regulate programmed cell death of rat microglia/brain macrophages in vitro. *J Neuroimmunol*, **63**, 55-61.

Gehrmann, J. & Banati, R.B. (1995) Microglial turnover in the injured CNS: activated microglia undergo delayed DNA fragmentation following peripheral nerve injury. *J Neuropathol Exp Neurol*, **54**, 680-688.

Gehrmann, J., Banati, R.B., Wiessner, C., Hossmann, K.A. & Kreutzberg, G.W. (1995) Reactive microglia in cerebral ischaemia: an early mediator of tissue damage? *Neuropathol Appl Neurobiol*, **21**, 277-289.

Gerfen, C.R. (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature*, **311**, 461-464.

Gerfen, C.R., Baimbridge, K.G. & Miller, J.J. (1985) The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proc Natl Acad Sci U S A*, **82**, 8780-8784.

Gerfen, C.R. (1985) The neostriatal mosaic. I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. *J Comp Neurol,* **236**, 454-476.

Gerfen, C.R. & Engber, T.M. (1992) Molecular neuroanatomic mechanisms of Parkinson's disease: a proposed therapeutic approach. *Neurol Clin,* **10**, 435-449.

Gerfen, C.R. (1992) The neostriatal mosaic: multiple levels of compartmental organization. *Trends Neurosci,* **15**, 133-139.

German, D.C. & Liang, C.L. (1993) Neuroactive peptides exist in the midbrain dopaminergic neurons that contain calbindin-D28k. *Neuroreport,* **4**, 491-494.

Ghosh, A., Carnahan, J. & Greenberg, M.E. (1994) Requirement for BDNF in activity-dependent survival of cortical neurons. *Science,* **263**, 1618-1623.

Giehl, K.M., Schutte, A., Mestres, P. & Yan, Q. (1998) The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons in vivo is mediated by an endogenous brain-derived neurotrophic factor mechanism. *J Neurosci,* **18**, 7351-7360.

Gimenez-Amaya, J.M. & Graybiel, A.M. (1990) Compartmental origins of the striatopallidal projection in the primate. *Neuroscience,* **34**, 111-126.

Giulian, D., Woodward, J., Young, D.G., Krebs, J.F. & Lachman, L.B. (1988) Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J Neurosci,* **8**, 2485-2490.

Goldberg, Y.P., Nicholson, D.W., Rasper, D.M., Kalchman, M.A., Koide, H.B., Graham, R.K., Bromm, M., Kazemi-Esfarjani, P., Thornberry, N.A., Vaillancourt, J.P. & Hayden, M.R. (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat Genet,* **13**, 442-449.

Gotz, R., Koster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M. & Thoenen, H. (1994) Neurotrophin-6 is a new member of the nerve growth factor family. *Nature*, **372**, 266-269.

Grau, V., Herbst, B., van der Meide, P.H. & Steiniger, B. (1997) Activation of microglial and endothelial cells in the rat brain after treatment with interferon-gamma in vivo. *Glia*, **19**, 181-189.

Graveland, G.A. & DiFiglia, M. (1985) The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. *Brain Res*, **327**, 307-311.

Graveland, G.A., Williams, R.S. & DiFiglia, M. (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. *Science*, **227**, 770-773.

Graveland, G.A., Williams, R.S. & DiFiglia, M. (1985) A Golgi study of the human neostriatum: neurons and afferent fibers. *J Comp Neurol*, **234**, 317-333.

Graybiel, A.M. & Ragsdale, C.W.J. (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *Proc Natl Acad Sci U S A*, **75**, 5723-5726.

Guilarte, T.R., Kuhlmann, A.C., O'Callaghan, J.P. & Miceli, R.C. (1995) Enhanced expression of peripheral benzodiazepine receptors in trimethyltin-exposed rat brain: a biomarker of neurotoxicity. *Neurotoxicology*, **16**, 441-450.

Gusella, J.F., MacDonald, M.E., Ambrose, C.M. & Duyao, M.P. (1993) Molecular genetics of Huntington's disease. *Arch Neurol*, **50**, 1157-1163.

Gusella, J.F. & MacDonald, M.E. (1993) Hunting for Huntington's disease. *Mol Genet Med*, **3**, 139-158.

Gutekunst, C.A., Levey, A.I., Heilman, C.J., Whaley, W.L., Yi, H., Nash, N.R., Rees, H.D., Madden, J.J. & Hersch, S.M. (1995) Identification and localization of

huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. *Proc Natl Acad Sci U S A*, **92**, 8710-8714.

Guyot, M.C., Hantraye, P., Dolan, R., Palfi, S., Maziere, M. & Brouillet, E. (1997) Quantifiable bradykinesia, gait abnormalities and Huntington's disease-like striatal lesions in rats chronically treated with 3-nitropropionic acid. *Neuroscience*, **79**, 45-56.

Hagg, T. (1998) Neurotrophins prevent death and differentially affect tyrosine hydroxylase of adult rat nigrostriatal neurons in vivo. *Exp Neurol*, **149**, 183-192.

Hallbook, F., Ebendal, T. & Persson, H. (1988) Production and characterization of biologically active recombinant beta nerve growth factor. *Mol Cell Biol*, **8**, 452-456.

Han, B.H., D'Costa, A., Back, S.A., Parsadanian, M., Patel, S., Shah, A.R., Gidday, J.M., Srinivasan, A., Deshmukh, M. & Holtzman, D.M. BDNF blocks caspase-3 activation in neonatal hypoxia-ischemia. *Neurobiol Dis 2000. Feb. ;7. (1.):38. -53.*, **7**, 38-53.

Haniu, M., Montestruque, S., Bures, E.J., Talvenheimo, J., Toso, R., Lewis-Sandy, S., Welcher, A.A. & Rohde, M.F. (1997) Interactions between brain-derived neurotrophic factor and the TRKB receptor. Identification of two ligand binding domains in soluble TRKB by affinity separation and chemical cross-linking. *J Biol Chem*, **272**, 25296-25303.

Harper, P.S. (1992) Huntington disease and the abuse of genetics. *Am J Hum Genet*, **50**, 460-464.

Harrington, K.A., Augood, S.J., Kingsbury, A.E., Foster, O.J. & Emson, P.C. (1996) Dopamine transporter (Dat) and synaptic vesicle amine transporter (VMAT2) gene expression in the substantia nigra of control and Parkinson's disease. *Brain Res Mol Brain Res*, **36**, 157-162.

Hefti, F., Knusel, B. & Lapchak, P.A. (1993) Protective effects of nerve growth factor and brain-derived neurotrophic factor on basal forebrain cholinergic neurons in adult rats with partial fimbrial transections. *Prog Brain Res,* **98**, 257-263.

Heikkila, R.E., Manzino, L., Cabbat, F.S. & Duvoisin, R.C. (1985) Studies on the oxidation of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase B. *J Neurochem,* **45**, 1049-1054.

Heikkila, R.E., Nicklas, W.J. & Duvoisin, R.C. (1985) Dopaminergic toxicity after the stereotaxic administration of the 1-methyl-4-phenylpyridinium ion (MPP+) to rats. *Neurosci Lett,* **59**, 135-140.

Heikkila, R.E., Manzino, L., Cabbat, F.S. & Duvoisin, R.C. (1985) Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and several of its analogues on the dopaminergic nigrostriatal pathway in mice. *Neurosci Lett,* **58**, 133-137.

Heinemann, U., Gabriel, S., Schuchmann, S. & Eder, C. (1999) Contribution of astrocytes to seizure activity. *Adv Neurol,* **79**, 583-590.

Hendry, I.A., Stockel, K., Thoenen, H. & Iversen, L.L. (1974) The retrograde axonal transport of nerve growth factor. *Brain Res,* **68**, 103-121.

Herkenham, M. & Pert, C.B. (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. *Nature,* **291**, 415-418.

Herrera-Marschitz, M. & Ungerstedt, U. (1984) Evidence that striatal efferents relate to different dopamine receptors. *Brain Res,* **323**, 269-278.

Herrero, M.T., Augood, S.J., Asensi, H., Hirsch, E.C., Agid, Y., Obeso, J.A. & Emson, P.C. (1996) Effects of L-DOPA-therapy on dopamine D2 receptor mRNA expression in the striatum of MPTP-intoxicated parkinsonian monkeys. *Brain Res Mol Brain Res,* **42**, 149-155.

Hohn, A., Leibrock, J., Bailey, K. & Barde, Y.A. (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature,* **344**, 339-341.

Hollmann, M. & Heinemann, S. (1994) Cloned glutamate receptors. *Annu Rev Neurosci,* **17**, 31-108.

Hontanilla, B., Parent, A. & Gimenez-Amaya, J.M. (1997) Parvalbumin and calbindin D-28k in the entopeduncular nucleus, subthalamic nucleus, and substantia nigra of the rat as revealed by double-immunohistochemical methods. *Synapse,* **25**, 359-367.

Huang, E.J., Wilkinson, G.A., Farinas, I., Backus, C., Zang, K., Wong, S.L. & Reichardt, L.F. (1999) Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC. *Development,* **126**, 2191-2203.

Huang, Q., Zhou, D., Sapp, E., Aizawa, H., Ge, P., Bird, E.D., Vonsattel, J.P. & DiFiglia, M. (1995) Quinolinic acid-induced increases in calbindin D28k immunoreactivity in rat striatal neurons in vivo and in vitro mimic the pattern seen in Huntington's disease. *Neuroscience,* **65**, 397-407.

Hung, H.C. & Lee, E.H. (1996) The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP+ toxicity: role of BDNF gene expression. *Brain Res Mol Brain Res,* **41**, 14-26.

Hyman, C., Hofer, M., Barde, Y.A., Juhasz, M., Yancopoulos, G.D., Squinto, S.P. & Lindsay, R.M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature,* **350**, 230-232.

Hyman, C., Juhasz, M., Jackson, C., Wright, P., Ip, N.Y. & Lindsay, R.M. (1994) Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *Journal of Neuroscience,* **14**, 335-347.

Hynes, M.A., Poulsen, K., Armanini, M., Berkemeier, L., Phillips, H. & Rosenthal, A. (1994) Neurotrophin-4/5 is a survival factor for embryonic midbrain dopaminergic neurons in enriched cultures. *J Neurosci Res*, **37**, 144-154.

Ibanez, C.F. (1994) Structure-function relationships in the neurotrophin family. *J Neurobiol*, **25**, 1349-1361.

Ibanez, C.F. (1995) Neurotrophic factors: from structure-function studies to designing effective therapeutics. *Trends Biotechnol*, **13**, 217-227.

Ibanez, C.F. (1996) Neurotrophin-4: the odd one out in the neurotrophin family. *Neurochem Res*, **21**, 787-793.

Imamoto, K. & Leblond, C.P. (1978) Radioautographic investigation of gliogenesis in the corpus callosum of young rats. II. Origin of microglial cells. *J Comp Neurol*, **180**, 139-163.

Ip, N.Y., Ibanez, C.F., Nye, S.H., McClain, J., Jones, P.F., Gies, D.R., Belluscio, L., Le Beau, M.M., Espinosa, R. & Squinto, S.P. (1992) Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. *Proc Natl Acad Sci U S A*, **89**, 3060-3064.

Irwin, I. & Langston, J.W. (1985) Selective accumulation of MPP+ in the substantia nigra: a key to neurotoxicity? *Life Sci*, **36**, 207-212.

Isaacs, K.R. & Jacobowitz, D.M. (1994) Mapping of the colocalization of calretinin and tyrosine hydroxylase in the rat substantia nigra and ventral tegmental area. *Exp Brain Res*, **99**, 34-42.

Jarvis, M.F. & Wagner, G.C. (1985) Neurochemical and functional consequences following 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) and methamphetamine. *Life Sci*, **36**, 249-254.

Javitch, J.A., D'Amato, R.J., Strittmatter, S.M. & Snyder, S.H. (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine: uptake of the

metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci U S A,* **82**, 2173-2177.

Jones, K.R., Farinas, I., Backus, C. & Reichardt, L.F. (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell,* **76**, 989-999.

Kaplan, D.R., Hempstead, B.L., Martin-Zanca, D., Chao, M.V. & Parada, L.F. (1991) The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science,* **252**, 554-558.

Kaplan, D.R., Martin-Zanca, D. & Parada, L.F. (1991) Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. *Nature,* **350**, 158-160.

Kato, H., Kogure, K., Araki, T. & Itoyama, Y. (1994) Astroglial and microglial reactions in the gerbil hippocampus with induced ischemic tolerance. *Brain Res,* **664**, 69-76.

Kato, H., Araki, T., Itoyama, Y., Kogure, K. & Kato, K. (1995) An immunohistochemical study of heat shock protein-27 in the hippocampus in a gerbil model of cerebral ischemia and ischemic tolerance. *Neuroscience,* **68**, 65-71.

Kawaguchi, Y. (1993) Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. *J Neurosci,* **13**, 4908-4923.

Kiefer, R. & Kreutzberg, G.W. (1991) Effects of dexamethasone on microglial activation in vivo: selective downregulation of major histocompatibility complex class II expression in regenerating facial nucleus. *J Neuroimmunol,* **34**, 99-108.

Kita, H., Kosaka, T. & Heizmann, C.W. (1990) Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. *Brain Res,* **536**, 1-15.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. & Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature,* **392**, 605-608.

Klein, R., Parada, L.F., Coulier, F. & Barbacid, M. (1989) trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. *EMBO J,* **8**, 3701-3709.

Klein, R., Nanduri, V., Jing, S.A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K.R., Reichardt, L.F. & Barbacid, M. (1991) The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell,* **66**, 395-403.

Klein, R., Lamballe, F., Bryant, S. & Barbacid, M. (1992) The trkB tyrosine protein kinase is a receptor for neurotrophin-4. *Neuron,* **8**, 947-956.

Klein, R., Smeyne, R.J., Wurst, W., Long, L.K., Auerbach, B.A., Joyner, A.L. & Barbacid, M. (1993) Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. *Cell,* **75**, 113-122.

Klein, R. (1994) Role of neurotrophins in mouse neuronal development. *FASEB J,* **8**, 738-744.

Knusel, B., Beck, K.D., Winslow, J.W., Rosenthal, A., Burton, L.E., Widmer, H.R., Nikolics, K. & Hefti, F. (1992) Brain-derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degenerative changes after axotomy in the adult rat brain. *J Neurosci,* **12**, 4391-4402.

Knusel, B. & Gao, H. (1996) Neurotrophins and Alzheimer's disease: beyond the cholinergic neurons. *Life Sci,* **58**, 2019-2027.

Koh, J.Y., Peters, S. & Choi, D.W. (1986) Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. *Science,* **234**, 73-76.

Koliatsos, V.E. & Price, D.L. (1996) Axotomy as an experimental model of neuronal injury and cell death. *Brain Pathol,* **6**, 447-465.

Kosinski, C.M., Cha, J.H., Young, A.B., Persichetti, F., MacDonald, M., Gusella, J.F., Penney, J.B.J. & Standaert, D.G. (1997) Huntingtin immunoreactivity in the rat

neostriatum: differential accumulation in projection and interneurons. *Exp Neurol*, **144**, 239-247.

Kreutzberg, G.W. (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci*, **19**, 312-318.

Kume, T., Kouchiyama, H., Kaneko, S., Maeda, T., Akaike, A., Shimohama, S., Kihara, T., Kimura, J., Wada, K. & Koizumi, S. (1997) BDNF prevents NO mediated glutamate cytotoxicity in cultured cortical neurons. *Brain Res*, **756**, 200-204.

Kurkowska-Jastrzebska, I., Wronska, A., Kohutnicka, M., Czlonkowski, A. & Czlonkowska, A. (1999) The inflammatory reaction following 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine intoxication in mouse. *Exp Neurol*, **156**, 50-61.

Lai, K.O., Fu, W.Y., Ip, F.C. & Ip, N.Y. (1998) Cloning and expression of a novel neurotrophin, NT-7, from carp. *Mol Cell Neurosci*, **11**, 64-76.

Lamballe, F., Klein, R. & Barbacid, M. (1991) trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. *Cell*, **66**, 967-979.

Lamballe, F., Klein, R. & Barbacid, M. (1991) The trk family of oncogenes and neurotrophin receptors. *Princess Takamatsu Symp*, **22**, 153-170.

Laming, P.R., Kimelberg, H., Robinson, S., Salm, A., Hawrylak, N., Muller, C., Roots, B. & Ng, K. (2000) Neuronal-glial interactions and behaviour. *Neurosci Biobehav Rev*, **24**, 295-340.

Landwehrmeyer, G.B., McNeil, S.M., Dure, L.S., Ge, P., Aizawa, H., Huang, Q., Ambrose, C.M., Duyao, M.P., Bird, E.D. & Bonilla, E. (1995) Huntington's disease gene: regional and cellular expression in brain of normal and affected individuals. *Ann Neurol*, **37**, 218-230.

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.

Langston, J.W., Forno, L.S., Rebert, C.S. & Irwin, I. (1984) Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. *Brain Res,* **292**, 390-394.

Langston, J.W. & Ballard, P. (1984) Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. *Can J Neurol Sci,* **11**, 160-165.

Langston, J.W., Langston, E.B. & Irwin, I. (1984) MPTP-induced parkinsonism in human and non-human primates--clinical and experimental aspects. *Acta Neurol Scand Suppl,* **100**, 49-54.

Lazarov-Spiegler, O., Solomon, A.S., Zeev-Brann, A.B., Hirschberg, D.L., Lavie, V. & Schwartz, M. (1996) Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J,* **10**, 1296-1302.

Leah, J.D., Herdegen, T., Murashov, A., Dragunow, M. & Bravo, R. (1993) Expression of immediate early gene proteins following axotomy and inhibition of axonal transport in the rat central nervous system. *Neuroscience,* **57**, 53-66.

Lees, G.J. (1993) The possible contribution of microglia and macrophages to delayed neuronal death after ischemia. *J Neurol Sci,* **114**, 119-122.

Lehrmann, E., Christensen, T., Zimmer, J., Diemer, N.H. & Finsen, B. (1997) Microglial and macrophage reactions mark progressive changes and define the penumbra in the rat neocortex and striatum after transient middle cerebral artery occlusion. *J Comp Neurol,* **386**, 461-476.

Lerea, L.S. & McNamara, J.O. (1993) Ionotropic glutamate receptor subtypes activate c-fos transcription by distinct calcium-requiring intracellular signaling pathways. *Neuron,* **10** , 31-41.

Levi-Montalcini, R. (1987) The nerve growth factor 35 years later. *Science,* **237**, 1154-1162.

Levi-Montalcini, R. (1987) The nerve growth factor: thirty-five years later. *EMBO J,* **6**, 1145-1154.

Li, S.H., Schilling, G., Young, W.S., Li, X.J., Margolis, R.L., Stine, O.C., Wagster, M.V., Abbott, M.H., Franz, M.L. & Ranen, N.G. (1993) Huntington's disease gene (IT15) is widely expressed in human and rat tissues. *Neuron,* **11**, 985-993.

Liang, C.L., Sinton, C.M. & German, D.C. (1996) Midbrain dopaminergic neurons in the mouse: co-localization with Calbindin-D28K and calretinin. *Neuroscience,* **75**, 523-533.

Lindholm, D. (1994) Role of neurotrophins in preventing glutamate induced neuronal cell death. *J Neurol,* **242**, S16-S18.

Lindsay, R.M. (1979) Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurones. *Nature,* **282**, 80-82.

Lindvall, O., Kokaia, Z., Bengzon, J., Elmer, E. & Kokaia, M. (1994) Neurotrophins and brain insults. *Trends Neurosci,* **17**, 490-496.

Ling, E.A. & Wong, W.C. (1993) The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia,* **7**, 9-18.

Lingor, P., Unsicker, K. & Krieglstein, K. GDNF and NT-4 protect midbrain dopaminergic neurons from toxic damage by iron and nitric oxide. *Exp Neurol 2000. May. ;163. (1.):55. -62.*, **163**, 55-62.

Lipton, S.A. & Rosenberg, P.A. (1994) Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med,* **330**, 613-622.

Liu, X., Ernfors, P., Wu, H. & Jaenisch, R. (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. *Nature,* **375**, 238-241.

Loughlin, A.J., Woodroofe, M.N. & Cuzner, M.L. (1992) Regulation of Fc receptor and major histocompatibility complex antigen expression on isolated rat microglia by

tumour necrosis factor, interleukin-1 and lipopolysaccharide: effects on interferon-gamma induced activation. *Immunology*, **75**, 170-175.

Ludwin, S.K. (1997) The pathobiology of the oligodendrocyte. *J Neuropathol Exp Neurol*, **56**, 111-124.

Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E., Lindsay, R.M. & Yancopoulos, G.D. (1990) NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron*, **5**, 501-509.

Maisonpierre, P.C., Belluscio, L., Squinto, S., Ip, N.Y., Furth, M.E., Lindsay, R.M. & Yancopoulos, G.D. (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science*, **247**, 1446-1451.

Maisonpierre, P.C., Le Beau, M.M., Espinosa, R., Ip, N.Y., Belluscio, L., de la Monte, S.M., Squinto, S., Furth, M.E. & Yancopoulos, G.D. (1991) Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. *Genomics*, **10**, 558-568.

Malach, R. & Graybiel, A.M. (1986) Mosaic architecture of the somatic sensory-recipient sector of the cat's striatum. *J Neurosci*, **6**, 3436-3458.

Maness, L.M., Kastin, A.J., Weber, J.T., Banks, W.A., Beckman, B.S. & Zadina, J.E. (1994) The neurotrophins and their receptors: structure, function, and neuropathology. *Neurosci Biobehav Rev*, **18**, 143-159.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W. & Bates, G.P. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell*, **87**, 493-506.

Marchis, S.D., Modena, C., Peretto, P., Migheli, A., Margolis, F.L. & Fasolo, A. (2000) Carnosine-related dipeptides in neurons and glia. *Biochemistry (Mosc)*, **65**, 824-833.

Markey, S.P., Johannessen, J.N., Chiueh, C.C., Burns, R.S. & Herkenham, M.A. (1984) Intraneuronal generation of a pyridinium metabolite may cause drug-induced parkinsonism. *Nature*, **311**, 464-467.

Martin-Zanca, D., Hughes, S.H. & Barbacid, M. (1986) A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature*, **319**, 743-748.

Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T. & Barbacid, M. (1989) Molecular and biochemical characterization of the human trk proto-oncogene. *Mol Cell Biol*, **9**, 24-33.

Martin, J.B. & Gusella, J.F. (1986) Huntington's disease. Pathogenesis and management. *N Engl J Med*, **315**, 1267-1276.

Martindale, D., Hackam, A., Wieczorek, A., Ellerby, L., Wellington, C., McCutcheon, K., Singaraja, R., Kazemi-Esfarjani, P., Devon, R., Kim, S.U., Bredesen, D.E., Tufaro, F. & Hayden, M.R. (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat Genet*, **18**, 150-154.

Martinez-Serrano, A. & Bjorklund, A. (1996) Protection of the neostriatum against excitotoxic damage by neurotrophin-producing, genetically modified neural stem cells. *J Neurosci*, **16**, 4604-4616.

Marty, S., Berzaghi, M. & Berninger, B. (1997) Neurotrophins and activity-dependent plasticity of cortical interneurons. *Trends Neurosci*, **20**, 198-202.

McAllister, A.K., Katz, L.C. & Lo, D.C. (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron*, **18**, 767-778.

McAllister, A.K. (1999) Subplate neurons: a missing link among neurotrophins, activity, and ocular dominance plasticity? *Proc Natl Acad Sci U S A*, **96**, 13600-13602.

McGeer, P.L. & McGeer, E.G. (1978) Intracerebral injections of kainic acid and tetanus toxin: possible models for the signs of chorea and dystonia. *Adv Neurol,* **21**, 331-338.

McGeer, P.L., Itagaki, S., Boyes, B.E. & McGeer, E.G. (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology,* **38**, 1285-1291.

McGeer, P.L., Kawamata, T., Walker, D.G., Akiyama, H., Tooyama, I. & McGeer, E.G. (1993) Microglia in degenerative neurological disease. *Glia,* **7**, 84-92.

McGraw, J., Hiebert, G.W. & Steeves, J.D. (2001) Modulating astrogliosis after neurotrauma. *J Neurosci Res,* **63**, 109-115.

McRitchie, D.A., Cartwright, H., Pond, S.M., van der Schyf, C.J., Castagnoli, N.J., van der Nest, D.G. & Halliday, G.M. (1998) The midbrain dopaminergic cell groups in the baboon Papio ursinus. *Brain Res Bull,* **47**, 611-623.

Merlio, J.P., Ernfors, P., Jaber, M. & Persson, H. (1992) Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience,* **51**, 513-532.

Middlemas, D.S., Lindberg, R.A. & Hunter, T. (1991) trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol Cell Biol,* **11**, 143-153.

Miranda, R.C., Sohrabji, F. & Toran-Allerand, C.D. (1993) Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. *Proc Natl Acad Sci U S A,* **90**, 6439-6443.

Mizuno, K., Carnahan, J. & Nawa, H. (1994) Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. *Dev Biol,* **165**, 243-256.

Moller, J.C., Klein, M.A., Haas, S., Jones, L.L., Kreutzberg, G.W. & Raivich, G. (1996) Regulation of thrombospondin in the regenerating mouse facial motor nucleus. *Glia,* **17**, 121-132.

Morens, D.M., White, L.R. & Davis, J.W. (1996) Re: "The frequency of idiopathic Parkinson's disease by age, ethnic group, and sex in northern Manhattan, 1988-1993&quot. *Am J Epidemiol,* **144**, 198-199.

Morton, A.J. & Leavens, W. (2000) Mice transgenic for the human Huntington's disease mutation have reduced sensitivity to kainic acid toxicity. *Brain Res Bull,* **52**, 51-59.

Myers, R., Manjil, L.G., Cullen, B.M., Price, G.W., Frackowiak, R.S. & Cremer, J.E. (1991) Macrophage and astrocyte populations in relation to [3H]PK 11195 binding in rat cerebral cortex following a local ischaemic lesion. *J Cereb Blood Flow Metab,* **11**, 314-322.

Nakai, M., Qin, Z., Wang, Y. & Chase, T.N. (2000) NMDA and non-NMDA receptor-stimulated IkappaB-alpha degradation: differential effects of the caspase-3 inhibitor DEVD.CHO, ethanol and free radical scavenger OPC-14117. *Brain Res,* **859**, 207-216.

Nakao, N., Kokaia, Z., Odin, P. & Lindvall, O. (1995) Protective effects of BDNF and NT-3 but not PDGF against hypoglycemic injury to cultured striatal neurons. *Exp Neurol,* **131**, 1-10.

Nakao, N., Brundin, P., Funa, K., Lindvall, O. & Odin, P. (1995) Trophic and protective actions of brain-derived neurotrophic factor on striatal DARPP-32-containing neurons in vitro. *Brain Res Dev Brain Res,* **90**, 92-101.

Neafsey, E.J., Albores, R., Gearhart, D., Kindel, G., Raikoff, K., Tamayo, F. & Collins, M.A. (1995) Methyl-beta-carbolinium analogs of MPP+ cause nigrostriatal toxicity after substantia nigra injections in rats. *Brain Res,* **675**, 279-288.

Nilsson, A.S., Fainzilber, M., Falck, P. & Ibanez, C.F. (1998) Neurotrophin-7: a novel member of the neurotrophin family from the zebrafish. *FEBS Lett,* **424**, 285-290.

Nishino, H., Fujimoto, I., Shimano, Y., Hida, H., Kumazaki, M. & Fukuda, A. (1996) 3-Nitropropionic acid produces striatum selective lesions accompanied by iNOS expression. *J Chem Neuroanat*, **10**, 209-212.

Nishino, H., Hida, H., Kumazaki, M., Shimano, Y., Nakajima, K., Shimizu, H., Ooiwa, T. & Baba, H. (2000) The striatum is the most vulnerable region in the brain to mitochondrial energy compromise: a hypothesis to explain its specific vulnerability. *J Neurotrauma*, **17**, 251-260.

Nordborg, C., Sokrab, T.E. & Johansson, B.B. (1994) Oedema-related tissue damage after temporary and permanent occlusion of the middle cerebral artery. *Neuropathol Appl Neurobiol*, **20**, 56-65.

Norton, W.T., Aquino, D.A., Hozumi, I., Chiu, F.C. & Brosnan, C.F. (1992) Quantitative aspects of reactive gliosis: a review. *Neurochem Res*, **17**, 877-885.

O'Callaghan, J.P. & Miller, D.B. (1988) Acute exposure of the neonatal rat to triethyltin results in persistent changes in neurotypic and gliotypic proteins. *J Pharmacol Exp Ther*, **244**, 368-378.

O'Callaghan, J.P., Miller, D.B. & Reinhard, J.F.J. (1990) Characterization of the origins of astrocyte response to injury using the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain Res*, **521**, 73-80.

O'Callaghan, J.P., Miller, D.B. & Reinhard, J.F.J. (1990) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage of striatal dopaminergic fibers attenuates subsequent astrocyte response to MPTP. *Neurosci Lett*, **117**, 228-233.

O'Callaghan, J.P. & Jensen, K.F. (1992) Enhanced expression of glial fibrillary acidic protein and the cupric silver degeneration reaction can be used as sensitive and early indicators of neurotoxicity. *Neurotoxicology*, **13**, 113-122.

Oertel, W.H., Tappaz, M.L., Berod, A. & Mugnaini, E. (1982) Two-color immunohistochemistry for dopamine and GABA neurons in rat substantia nigra and zona incerta. *Brain Res Bull*, **9**, 463-474.

Oertel, W.H. & Mugnaini, E. (1984) Immunocytochemical studies of GABAergic neurons in rat basal ganglia and their relations to other neuronal systems. *Neurosci Lett*, **47**, 233-238.

Olney, J.W. (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science*, **164**, 719-721.

Ona, V.O., Li, M., Vonsattel, J.P., Andrews, L.J., Khan, S.Q., Chung, W.M., Frey, A.S., Menon, A.S., Li, X.J., Stieg, P.E., Yuan, J., Penney, J.B., Young, A.B., Cha, J.H. & Friedlander, R.M. (1999) Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature*, **399**, 263-267.

Pappas, I.S. & Parnavelas, J.G. (1997) Neurotrophins and basic fibroblast growth factor induce the differentiation of calbindin-containing neurons in the cerebral cortex. *Exp Neurol*, **144**, 302-314.

Parent, A. & Hazrati, L.N. (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res Brain Res Rev*, **20**, 91-127.

Parpura, V. & Haydon, P.G. (2000) From the cover: physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc Natl Acad Sci U S A*, **97**, 8629-8634.

Pennypacker, K.R., Kassed, C.A., Eidizadeh, S. & O'Callaghan, J.P. (2000) Brain injury: prolonged induction of transcription factors. *Acta Neurobiol Exp (Warsz)*, **60**, 515-530.

Perez-Navarro, E., Alberch, J., Neveu, I. & Arenas, E. (1999) Brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5 differentially regulate the phenotype and prevent degenerative changes in striatal projection neurons after excitotoxicity in vivo. *Neuroscience*, **91**, 1257-1264.

Perez-Navarro, E., Akerud, P., Marco, S., Canals, J.M., Tolosa, E., Arenas, E. & Alberch, J. (2000) Neurturin protects striatal projection neurons but not interneurons in a rat model of Huntington's disease. *Neuroscience*, **98**, 89-96.

Perry, V.H. & Gordon, S. (1988) Macrophages and microglia in the nervous system. *Trends Neurosci,* **11**, 273-277.

Peters, A., Josephson, K. & Vincent, S.L. (1991) Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex. *Anat Rec,* **229**, 384-398.

Petersen, A., Mani, K. & Brundin, P. (1999) Recent advances on the pathogenesis of Huntington's disease. *Exp Neurol,* **157**, 1-18.

Plaitakis, A. & Shashidharan, P. (2000) Glutamate transport and metabolism in dopaminergic neurons of substantia nigra: implications for the pathogenesis of Parkinson's disease. *J Neurol,* **247 Suppl 2**, II25-II35.

Planas, A.M., Justicia, C., Soriano, M.A. & Ferrer, I. (1998) Epidermal growth factor receptor in proliferating reactive glia following transient focal ischemia in the rat brain. *Glia,* **23**, 120-129.

Plumier, J.C., Armstrong, J.N., Landry, J., Babity, J.M., Robertson, H.A. & Currie, R.W. (1996) Expression of the 27,000 mol. wt heat shock protein following kainic acid-induced status epilepticus in the rat. *Neuroscience,* **75**, 849-856.

Plumier, J.C., Hopkins, D.A., Robertson, H.A. & Currie, R.W. (1997) Constitutive expression of the 27-kDa heat shock protein (Hsp27) in sensory and motor neurons of the rat nervous system. *J Comp Neurol,* **384**, 409-428.

Plumier, J.C., David, J.C., Robertson, H.A. & Currie, R.W. (1997) Cortical application of potassium chloride induces the low-molecular weight heat shock protein (Hsp27) in astrocytes. *J Cereb Blood Flow Metab,* **17**, 781-790.

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I. & Nussbaum, R.L. (1997) Mutation in the alpha-

synuclein gene identified in families with Parkinson's disease. *Science,* **276**, 2045-2047.

Porter, J.T. & McCarthy, K.D. (1996) Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J Neurosci,* **16**, 5073-5081.

Qin, Z.H., Chen, R.W., Wang, Y., Nakai, M., Chuang, D.M. & Chase, T.N. (1999) Nuclear factor kappaB nuclear translocation upregulates c-Myc and p53 expression during NMDA receptor-mediated apoptosis in rat striatum. *J Neurosci,* **19**, 4023-4033.

Raghupathi, R., Graham, D.I. & McIntosh, T.K. (2000) Apoptosis after traumatic brain injury. *J Neurotrauma,* **17**, 927-938.

Ragsdale, C.W.J. & Graybiel, A.M. (1981) The fronto-striatal projection in the cat and monkey and its relationship to inhomogeneities established by acetylcholinesterase histochemistry. *Brain Res,* **208**, 259-266.

Ragsdale, C.W.J. & Graybiel, A.M. (1990) A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. *Proc Natl Acad Sci U S A,* **87**, 6196-6199.

Ramsay, R.R., Krueger, M.J., Youngster, S.K. & Singer, T.P. (1991) Evidence that the inhibition sites of the neurotoxic amine 1-methyl-4-phenylpyridinium (MPP+) and of the respiratory chain inhibitor piericidin A are the same. *Biochem J,* **273(Pt 2)**, 481-484.

Reiner, A. & Anderson, K.D. (1990) The patterns of neurotransmitter and neuropeptide co-occurrence among striatal projection neurons: conclusions based on recent findings. *Brain Res Brain Res Rev,* **15**, 251-265.

Resibois, A. & Rogers, J.H. (1992) Calretinin in rat brain: an immunohistochemical study. *Neuroscience,* **46**, 101-134.

Reynolds, D.S. & Morton, A.J. (1998) Changes in blood-brain barrier permeability following neurotoxic lesions of rat brain can be visualised with trypan blue. *J Neurosci Methods,* **79**, 115-121.

Ribak, C.E., Vaughn, J.E. & Roberts, E. (1979) The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. *J Comp Neurol,* **187**, 261-283.

Rio, C., Perez-Cerda, F., Matute, C. & Nieto-Sampedro, M. (1995) Preparation of a monoclonal antibody to a glycidic epitope of the epidermal growth factor receptor that recognizes inhibitors of astrocyte proliferation and reactive microglia. *J Neurosci Res,* **40**, 776-786.

Rocamora, N., Garcia-Ladona, F.J., Palacios, J.M. & Mengod, G. (1993) Differential expression of brain-derived neurotrophic factor, neurotrophin-3, and low-affinity nerve growth factor receptor during the postnatal development of the rat cerebellar system. *Brain Res Mol Brain Res,* **17**, 1-8.

Rocamora, N., Massieu, L., Boddeke, H.W., Palacios, J.M. & Mengod, G. (1994) Differential regulation of the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 mRNAs in adult rat brain after intrahippocampal injection of quinolinic acid. *Brain Res Mol Brain Res,* **26**, 89-98.

Rogers, J.H. (1992) Immunohistochemical markers in rat brain: colocalization of calretinin and calbindin-D28k with tyrosine hydroxylase. *Brain Res,* **587**, 203-210.

Rogove, A.D. & Tsirka, S.E. (1998) Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. *Curr Biol,* **8**, 19-25.

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.

Schlessinger, J. & Ullrich, A. (1992) Growth factor signaling by receptor tyrosine kinases. *Neuron,* **9**, 383-391.

Schmitt, I., Bachner, D., Megow, D., Henklein, P., Hameister, H., Epplen, J.T. & Riess, O. (1995) Expression of the Huntington disease gene in rodents: cloning the rat homologue and evidence for downregulation in non-neuronal tissues during development. *Hum Mol Genet,* **4**, 1173-1182.

Schneider, R. & Schweiger, M. (1991) A novel modular mosaic of cell adhesion motifs in the extracellular domains of the neurogenic trk and trkB tyrosine kinase receptors. *Oncogene,* **6**, 1807-1811.

Schneider, R. & Schweiger, M. (1991) The yeast DNA repair proteins RAD1 and RAD7 share similar putative functional domains. *FEBS Lett,* **283**, 203-206.

Schoepp, D.D. & Sacaan, A.I. (1994) Metabotropic glutamate receptors and neuronal degenerative disorders. *Neurobiol Aging,* **15**, 261-263.

Schutte, A., Yan, Q., Mestres, P. & Giehl, K.M. (2000) The endogenous survival promotion of axotomized rat corticospinal neurons by brain-derived neurotrophic factor is mediated via paracrine, rather than autocrine mechanisms. *Neurosci Lett,* **290**, 185-188.

Segal, R.A. & Greenberg, M.E. (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu Rev Neurosci,* **19**, 463-489.

Seroogy, K.B., Dangaran, K., Lim, S., Haycock, J.W. & Fallon, J.H. (1989) Ventral mesencephalic neurons containing both cholecystokinin- and tyrosine hydroxylase-like immunoreactivities project to forebrain regions. *J Comp Neurol,* **279**, 397-414.

Seroogy, K.B. & Gall, C.M. (1993) Expression of neurotrophins by midbrain dopaminergic neurons. *Exp Neurol,* **124**, 119-128.

Sharp, A.H., Loev, S.J., Schilling, G., Li, S.H., Li, X.J., Bao, J., Wagster, M.V., Kotzuk, J.A., Steiner, J.P. & Lo, A. (1995) Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron*, **14**, 1065-1074.

Shimohama, S., Tamura, Y., Akaike, A., Tsukahara, T., Ohara, O., Watanabe, S. & Kimura, J. (1993) Brain-derived neurotrophic factor pretreatment exerts a partially protective effect against glutamate-induced neurotoxicity in cultured rat cortical neurons. *Neurosci Lett*, **164**, 55-58.

Shults, C.W., Kimber, T. & Altar, C.A. (1995) BDNF attenuates the effects of intrastriatal injection of 6-hydroxydopamine. *Neuroreport*, **6**, 1109-1112.

Smith, Y. & Parent, A. (1986) Neuropeptide Y-immunoreactive neurons in the striatum of cat and monkey: morphological characteristics, intrinsic organization and co-localization with somatostatin. *Brain Res*, **372**, 241-252.

Smith, Y., Bevan, M.D., Shink, E. & Bolam, J.P. (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience*, **86**, 353-387.

Snell, R.G., MacMillan, J.C., Cheadle, J.P., Fenton, I., Lazarou, L.P., Davies, P., MacDonald, M.E., Gusella, J.F., Harper, P.S. & Shaw, D.J. (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat Genet*, **4**, 393-397.

Snell, R.G., Doucette-Stamm, L.A., Gillespie, K.M., Taylor, S.A., Riba, L., Bates, G.P., Altherr, M.R., MacDonald, M.E., Gusella, J.F. & Wasmuth, J.J. (1993) The isolation of cDNAs within the Huntington disease region by hybridisation of yeast artificial chromosomes to a cDNA library. *Hum Mol Genet*, **2**, 305-309.

Soppet, D., Escandon, E., Maragos, J., Middlemas, D.S., Reid, S.W., Blair, J., Burton, L.E., Stanton, B.R., Kaplan, D.R. & Hunter, T. (1991) The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell*, **65**, 895-903.

Squinto, S.P., Stitt, T.N., Aldrich, T.H., Davis, S., Bianco, S.M., Radziejewski, C., Glass, D.J., Masiakowski, P., Furth, M.E. & Valenzuela, D.M. (1991) trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell,* **65**, 885-893.

Steeves, J.D. & Tetzlaff, W. (1998) Engines, accelerators, and brakes on functional spinal cord repair. *Ann N Y Acad Sci,* **860**, 412-424.

Steranka, L.R. & Rhind, A.W. (1987) Effect of cysteine on the persistent depletion of brain monoamines by amphetamine, p-chloroamphetamine and MPTP. *Eur J Pharmacol,* **133**, 191-197.

Stine, O.C., Li, S.H., Pleasant, N., Wagster, M.V., Hedreen, J.C. & Ross, C.A. (1995) Expression of the mutant allele of IT-15 (the HD gene) in striatum and cortex of Huntington's disease patients. *Hum Mol Genet,* **4**, 15-18.

Stoll, G., Jander, S. & Schroeter, M. (1998) Inflammation and glial responses in ischemic brain lesions. *Prog Neurobiol,* **56**, 149-171.

Streit, W.J. & Kreutzberg, G.W. (1988) Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. *J Comp Neurol,* **268**, 248-263.

Streit, W.J., Graeber, M.B. & Kreutzberg, G.W. (1988) Functional plasticity of microglia: a review. *Glia,* **1**, 301-307.

Streit, W.J., Graeber, M.B. & Kreutzberg, G.W. (1989) Peripheral nerve lesion produces increased levels of major histocompatibility complex antigens in the central nervous system. *J Neuroimmunol,* **21**, 117-123.

Streit, W.J., Walter, S.A. & Pennell, N.A. (1999) Reactive microgliosis. *Prog Neurobiol,* **57**, 563-581.

Streit, W.J. (2000) Microglial response to brain injury: a brief synopsis. *Toxicol Pathol,* **28**, 28-30.

Strong, T.V., Tagle, D.A., Valdes, J.M., Elmer, L.W., Boehm, K., Swaroop, M., Kaatz, K.W., Collins, F.S. & Albin, R.L. (1993) Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nat Genet,* **5**, 259-265.

Studer, L., Spenger, C., Seiler, R.W., Altar, C.A., Lindsay, R.M. & Hyman, C. (1995) Comparison of the effects of the neurotrophins on the morphological structure of dopaminergic neurons in cultures of rat substantia nigra. *Eur J Neurosci,* **7**, 223-233.

Sun, C.J., Johannessen, J.N., Gessner, W., Namura, I., Singhaniyom, W., Brossi, A. & Chiueh, C.C. (1988) Neurotoxic damage to the nigrostriatal system in rats following intranigral administration of MPDP+ and MPP+. *J Neural Transm,* **74**, 75-86.

Suzumura, A., Sawada, M., Yamamoto, H. & Marunouchi, T. (1990) Effects of colony stimulating factors on isolated microglia in vitro. *J Neuroimmunol,* **30**, 111-120.

Takagi, H., Somogyi, P., Somogyi, J. & Smith, A.D. (1983) Fine structural studies on a type of somatostatin-immunoreactive neuron and its synaptic connections in the rat neostriatum: a correlated light and electron microscopic study. *J Comp Neurol,* **214**, 1-16.

Takeda, A., Onodera, H., Yamasaki, Y., Furukawa, K., Kogure, K., Obinata, M. & Shibahara, S. (1992) Decreased expression of neurotrophin-3 mRNA in the rat hippocampus following transient forebrain ischemia. *Brain Res,* **569**, 177-180.

Takei, N., Tanaka, O., Endo, Y., Lindholm, D. & Hatanaka, H. (1999) BDNF and NT-3 but not CNTF counteract the Ca2+ ionophore-induced apoptosis of cultured cortical neurons: involvement of dual pathways. *Neuropharmacology,* **38**, 283-288.

Tamatani, M., Ogawa, S. & Tohyama, M. (1998) Roles of Bcl-2 and caspases in hypoxia-induced neuronal cell death: a possible neuroprotective mechanism of peptide growth factors. *Brain Res Mol Brain Res,* **58**, 27-39.

Thoenen, H., Barde, Y.A., Davies, A.M. & Johnson, J.E. (1987) Neurotrophic factors and neuronal death. *Ciba Found Symp,* **126**, 82-95.

Timmusk, T., Belluardo, N., Metsis, M. & Persson, H. (1993) Widespread and developmentally regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. *Eur J Neurosci,* **5**, 605-613.

Tremblay, R., Hewitt, K., Lesiuk, H., Mealing, G., Morley, P. & Durkin, J.P. (1999) Evidence that brain-derived neurotrophic factor neuroprotection is linked to its ability to reverse the NMDA-induced inactivation of protein kinase C in cortical neurons. *J Neurochem,* **72**, 102-111.

Trottier, Y., Biancalana, V. & Mandel, J.L. (1994) Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset. *J Med Genet,* **31**, 377-382.

Trottier, Y., Lutz, Y., Stevanin, G., Imbert, G., Devys, D., Cancel, G., Saudou, F., Weber, C., David, G. & Tora, L. (1995) Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature,* **378**, 403-406.

Trottier, Y., Devys, D., Imbert, G., Saudou, F., An, I., Lutz, Y., Weber, C., Agid, Y., Hirsch, E.C. & Mandel, J.L. (1995) Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nat Genet,* **10**, 104-110.

Tseng, J.L., Baetge, E.E., Zurn, A.D. & Aebischer, P. (1997) GDNF reduces drug-induced rotational behavior after medial forebrain bundle transection by a mechanism not involving striatal dopamine. *J Neurosci,* **17**, 325-333.

Tsoulfas, P., Soppet, D., Escandon, E., Tessarollo, L., Mendoza-Ramirez, J.L., Rosenthal, A., Nikolics, K. & Parada, L.F. (1993) The rat trkC locus encodes multiple neurogenic receptors that exhibit differential response to neurotrophin-3 in PC12 cells. *Neuron,* **10**, 975-990.

Tsoulfas, P., Stephens, R.M., Kaplan, D.R. & Parada, L.F. (1996) TrkC isoforms with inserts in the kinase domain show impaired signaling responses. *J Biol Chem,* **271**, 5691-5697.

Tzourio, C., Rocca, W.A., Breteler, M.M., Baldereschi, M., Dartigues, J.F., Lopez-Pousa, S., Manubens-Bertran, J.M. & Alperovitch, A. (1997) Smoking and Parkinson's disease. An age-dependent risk effect? The EUROPARKINSON Study Group. *Neurology*, **49**, 1267-1272.

Ullrich, A., Gray, A., Berman, C., Coussens, L. & Dull, T.J. (1983) Sequence homology of human and mouse beta-NGF subunit genes. *Cold Spring Harb Symp Quant Biol*, **48 Pt 1**, 435-442.

Ultsch, M.H., Wiesmann, C., Simmons, L.C., Henrich, J., Yang, M., Reilly, D., Bass, S.H. & de Vos, A.M. (1999) Crystal structures of the neurotrophin-binding domain of TrkA, TrkB and TrkC. *J Mol Biol*, **290**, 149-159.

Ungerstedt, U. (1971) Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol Scand Suppl*, **367**, 95-122.

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

Ungerstedt, U. (1971) Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta Physiol Scand Suppl*, **367**, 49-68.

Urfer, R., Tsoulfas, P., O'Connell, L., Shelton, D.L., Parada, L.F. & Presta, L.G. (1995) An immunoglobulin-like domain determines the specificity of neurotrophin receptors. *EMBO J*, **14**, 2795-2805.

Varon, S., Nomura, J. & Shooter, E.M. (1967) The isolation of the mouse nerve growth factor protein in a high molecular weight form. *Biochemistry*, **6**, 2202-2209.

Varon, S., Nomura, J. & Shooter, E.M. (1967) Subunit structure of a high-molecular-weight form of the nerve growth factor from mouse submaxillary gland. *Proc Natl Acad Sci U S A*, **57**, 1782-1789.

Venero, J.L., Revuelta, M., Cano, J. & Machado, A. (1997) Time course changes in the dopaminergic nigrostriatal system following transection of the medial forebrain bundle: detection of oxidatively modified proteins in substantia nigra. *J Neurochem,* **68**, 2458-2468.

Ventimiglia, R., Mather, P.E., Jones, B.E. & Lindsay, R.M. (1995) The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. *Eur J Neurosci,* **7**, 213-222.

von Bartheld, C.S., Williams, R., Lefcort, F., Clary, D.O., Reichardt, L.F. & Bothwell, M. (1996) Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. *J Neurosci,* **16**, 2995-3008.

Walz, W. (2000) Controversy surrounding the existence of discrete functional classes of astrocytes in adult gray matter. *Glia,* **31**, 95-103.

Watkins, J.C. & Evans, R.H. (1981) Excitatory amino acid transmitters. *Annu Rev Pharmacol Toxicol,* **21**, 165-204.

Widmer, H.R. & Hefti, F. (1994) Neurotrophin-4/5 promotes survival and differentiation of rat striatal neurons developing in culture. *Eur J Neurosci,* **6**, 1669-1679.

Widmer, H.R. & Hefti, F. (1994) Stimulation of GABAergic neuron differentiation by NT-4/5 in cultures of rat cerebral cortex. *Brain Res Dev Brain Res,* **80**, 279-284.

Wiesmann, C., Ultsch, M.H., Bass, S.H. & de Vos, A.M. (1999) Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature,* **401**, 184-188.

Wilson, C.J. & Groves, P.M. (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular inject of horseradish peroxidase. *J Comp Neurol,* **194**, 599-615.

Wilson, M.A. & Molliver, M.E. (1994) Microglial response to degeneration of serotonergic axon terminals. *Glia,* **11**, 18-34.

Yao, L., Zhang, D. & Bernd, P. (1994) The onset of neurotrophin and trk mRNA expression in early embryonic tissues of the quail. *Dev Biol*, **165**, 727-730.

Yermakova, A. & O'Banion, M.K. (2000) Cyclooxygenases in the central nervous system: implications for treatment of neurological disorders. *Curr Pharm Des*, **6**, 1755-1776.

Young, A.B., Greenamyre, J.T., Hollingsworth, Z., Albin, R., D'Amato, C., Shoulson, I. & Penney, J.B. (1988) NMDA receptor losses in putamen from patients with Huntington's disease. *Science*, **241**, 981-983.

Yuen, E.C. & Mobley, W.C. (1996) Therapeutic potential of neurotrophic factors for neurological disorders. *Ann Neurol*, **40**, 346-354.

Zha, H., Remmers, E.F., Du, Y., Goldmuntz, E.A., Mathern, P., Zhang, H., Cash, J.M., Crofford, L.J. & Wilder, R.L. (1994) A single linkage group comprising 11 polymorphic DNA markers on rat chromosome 3. *Mamm. Genome*, **5**, 538-541.

Zhang, S.C. & Fedoroff, S. (1996) Neuron-microglia interactions in vitro. *Acta Neuropathol (Berl)*, **91**, 385-395.