Instituto Juan March
de Estudios e Investigaciones

155 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Neuronal Degeneration and Novel Therapeutic Approaches in Parkinson´s Disease

Organized by

C. W. Olanow, J. A. Obeso and R. Moratalla

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P. Brundin
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Session 1: The basal ganglia and the parkinsonian state: Molecular and physiological characteristics
Chair: José A. Obeso

Psychic disorders, basal ganglia, and Parkinson's disease

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Parkinson's disease is characterized classically by the progressive development of motor symptoms (bradykinesia, plastic rigidity, rest tremor) with an asymetric onset, well responding to levodopa treatment. Parkinsonian patients also present intellectuel (from executive dysfunction to dementia) and affective (from anxiety and depression to confusional states and hallucinations) disorders. As a result of recent discoveries concerning the anatomo-functional organization of the basal ganglia and cerebral cortex, the mechanisms of psychic disorders in patients start to be elucidated, including: nigro-striatal and extra-striatal dopaminergic dysfunction; dopaminergic and non-dopaminergic lesions; subcortical and cortical involvement; degenerative and non-degenerative cell loss. Parkinson's disease therefore appears as a heuristic model to understand human psychopathology. Conversely, the pathophysiology of mental disorders in Parkinson's disease allows to approach the role of basal ganglia dysfunction in the occurrence in affective disorders. This will be illustrated by showing the implication of limbic circuits of the pallidum, the subthalamic nucleus, the substantia nigra and the thalamus in various psychiatric disorders.

Achievements and challenges in the treatment of Parkinson's disease

Anthony E. Lang

University of Toronto

Achievements in Parkinson's disease have been many. The discovery of the underlying dopamine deficiency lead to the introduction of levodopa with profound effects on quality of life and even longevity. The unexpected development of motor complications has remained a therapeutic challenge. However, important advances have occurred in our understanding of the pathogenesis of these problems and a number of treatments have been introduced that can improve dyskinesias and especially fluctuations once they have developed. Strategies designed to provide more continuous dopaminergic stimulation may partially reverse pathogenetic mechanisms underlying motor complications and such treatment applied at the outset of symptomatic therapy may delay their onset. Advances in our understanding of basal ganglia physiology, including an appreciation for the important role of the subthalamic nucleus (STN), have been critical to the development of modern functional neurosurgical techniques. These have had a profound impact on the late-stage problems of severe motor fluctuations and dyskinesias and some patients who maintain an otherwise good response to levodopa may be returned to a near normal lifestyle. Advances in the fields of cellular therapies, trophic factors and gene therapies are also beginning to be applied in early clinical trials. Just as with the introduction of levodopa, new, sometimes unexpected, therapeutic challenges have arisen, for example the development of novel psychiatric and behavioral symptoms in patients treated with STN deep brain stimulation, the occurrence of dyskinesias complicating human fetal mesencephalic transplantation and the development of malignancies with gene therapies applied for other indications. Advances in the understanding of the possible pathogenesis of PD have come from discoveries of environmental and genetic causes of parkinsonism including important roles for mitochondrial dysfunction, reactive oxygen species and most recently the proteasomal-ubiquitin system and protein aggregation. Critical challenges remain. To date, no treatment has had a clear impact on the progressive neurodegenerative process. A related challenge is the need to establish effective surrogate disease state markers that can help unequivocally define neuroprotective effects of experimental interventions. A crucial challenge relates to the widely recognized but often neglected fact that Parkinson's disease is truly a "multisystems" degeneration which may only affect the substantia nigra well after it has taken hold in a variety of other lower brainstem sites as well as limbic and olfactory regions. It is this widespread neurodegeneration that accounts for the broad spectrum of levodopa-resistant symptoms (e.g., motor, behavioral, cognitive, sleep-related, sensory, autonomic) that become increasingly prominent as the disease progresses and that now constitute the greatest challenge to symptomatic therapy. The pattern and distribution of neurodegeneration, beyond the nigrostriatal dopaminergic system, highlights the urgent need for advances in our understanding of the pathogenesis of PD and with that the discovery of effective neuroprotective treatment. It is critical that this multisystems nature of the neurodegeneration is taken into account in the development and application of novel restorative/regenerative therapies.

Organization of the basal ganglia, Parkinson's disease and dyskinesias

Mahlon R. DeLong

Movement disorders are associated with increased and disordered discharge and synchronization in motor areas of the basal ganglia-thalamocortical loops. A role of brainstem projections from the internal pallidum and the substantia nigra, pars reticulate to the pedunculopontine nucleus (PPN) is also also suggested by recent studies. Several issues remain controversial, including the degree of segregation within and between the basal ganglia-thalamocortical loops, the scheme of "direct" and "indirect" striatal output pathways, the role of extrastriatal dopamine, and the mechanism of action of ablation and chronic electrical stimulation of the pallidum, subthalamic nucleus, and thalamus for movement disorders. Neuronal recording data and the observed effects of pallidal and thalamic lesions in these disorders suggests that the neuronal basis for the different basal ganglia movement disorders is not just changes in discharge rate, but also of altered discharge patterns, abnormal and excessive synchronization of discharge, altered proprioceptive feedback, and the appearance of increased "noise" in the basal ganglia output signal. In Parkinson's disease there is strong evidence for abnormal synchronized bursting and oscillations and increased cross-correlations between individual neurons in the pallidum and subthalamic nucleus. The motor circuit in Parkinson's has in a sense been "hijacked." In hyperkinetic disorders there is less direct evidence for changes in pattern and synchronization but it is clear that differences in rate alone cannot explain the development of these disorders. The effectiveness of ablative procedures and chronic stimulation in treating both hypo- and hyperkinetic disorders argues against a specific effect of these procedures on the pathophysiologic processes per se. Instead, it is more likely that these interventions remove the or replace with a more tolerable signal, the abnormal and disruptive signals directed to the thalamus, cortex and brainstem, thus allowing the otherwise relatively intact systems to compensate more efficiently and effectively.

Basal ganglia 'loops' with motor and non-motor areas of the cerebral cortex: a neural substrate to influence movement, cognition and perception

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Which cortical areas are the target of basal ganglia output? In the past, the answer to this question seemed quite simple. Efferents from the output nuclei of the basal ganglia were thought to terminate in a single region of the ventrolateral thalamus and influence a single cortical area, the primary motor cortex. Based on this view, the basal ganglia were believed to be exclusively motor structures involved in the generation and control of movement.

Over the past 20 years, an accumulation of information about the basal ganglia has led many investigators to challenge this view. Alexander, DeLong and Strick ('86) proposed that the output of the basal ganglia targeted specific areas of prefrontal, cingulate and orbital frontal cortex, as well as skeletomotor and oculomotor areas. If true, these circuits would provide the basal ganglia with the ability to influence not only motor control, but also cognitive and limbic function.

Until recently, it has been difficult to evaluate the validity of this and other proposals because of the problems inherent in tracing the multi-synaptic circuits which interconnect the basal ganglia with the cerebral cortex. To overcome some of the limitations of conventional tracers for circuit analysis, we developed the use of neurotropic viruses as transneuronal tracers in the central nervous system of primates (Strick and Card, '92; Kelly and Strick, '00). When specific strains of herpes simplex virus type 1 (HSV1) or rabies virus are injected into the cerebral cortex, the virus is taken up and transported transneuronally in the retrograde direction to label chains of synaptically interconnected neurons. With the appropriate adjustment of survival time, we can identify neurons linked to the injection site by one, two or even three synapses.

We have used virus tracing to examine the organization of basal ganglia-thalamocortical pathways to selected motor (Hoover and Strick, '93,'99), prefrontal (Middleton and Strick, '94,''02), inferotemporal (Middleton and Strick, '96) and posterior parietal areas of cortex (Clower et al., '01). The results of these studies indicate that each of these cortical areas is the target of basal ganglia output. Thus, it is now clear that the output from the basal ganglia influences more widespread regions of the cerebral cortex than previously suspected. These connections provide a neural substrate for the involvement of the basal ganglia in cognitive and perceptual processes such as working memory, rule-based learning, switching attention, visual perception and the planning of future behavior. Likewise, abnormal activity in basal ganglia loops with non-motor areas of the cortex could be the basis for the broad range of neuropsychiatric symptoms associated with basal ganglia disorders like Parkinson's and Huntington's Disease.

The output stage of basal ganglia processing displays a surprising degree of topographic organization. In fact, we have proposed that localized regions of the globus pallidus and substantia nigra pars reticulata form distinct output channels with each channel directed at a different cortical area. As the data on these circuits has accumulated, a general rule has emerged– namely, each cortical area that projects to the input stage of basal ganglia processing appears to receive (via the thalamus) efferents from the output stage of basal ganglia processing. This rule implies that multiple closed loops with the cerebral cortex represent a fundamental unit of basal ganglia circuitry.

In recent experiments we have used retrograde transneuronal transport of rabies virus to reveal third-order connections between areas of the cerebral cortex and the basal ganglia. In addition to confirming the presence of closed-loop circuits, these experiments revealed the existence of an open loop connection between a region of the ventral putamen and the primary motor cortex (Kelly and Strick, in press). The region of the ventral putamen that participates in this circuit receives input from the amygdala. Thus, this pathway may provide the limbic system with access to M1.

In summary, the basal ganglia can participate in two types of functional circuits with the cerebral cortex. One type involves multiple, parallel, closed-loops. These loops interconnect the basal ganglia with a broad and diverse set of cortical areas. Thus, they operate both inside and outside the domain of motor control. In addition, the ventral putamen participates in an open-loop circuit with the primary motor cortex. Whether a similar open-loop architecture also exists for other regions of the ventral striatum and non-motor areas of cortex remains to be determined. However, the ventral putamen connection with the primary motor cortex may represent an important route for interactions between the limbic system and the motor system.

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Session 2: Origin and progression of Parkinson's disease
Chair: Andres M. Lozano

Different roles of D1 and D2 receptor subtypes in levodopa-induced dyskinesias in a mouse model of Parkinson disease

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Treatment with L-DOPA is today the most efficacious, non invasive therapy for Parkinson's disease. However, chronic treatment with L-DOPA induces in most of the patients the appearance of abnormal involuntary movements known as dyskinesias. The molecular mechanisms underlying these abnormal movements are unknown, although the implication of dopamine receptors as well as other neurotransmitter receptors interacting with the dopaminergic system is suspected. In the present paper, we have studied the contribution of D1 and D2 receptor subtypes in levodopa-induced dyskinesias using knock out mice lacking D1 or D2 receptor in a mouse model of dyskinesia. In this experiment dyskinesias were induced with intermittent doses of L-DOPA in unilateral 6-OHDA-lesioned mice. In wild-type animals chronic treatment with L-DOPA induced abnormal involuntary movements including horizontal or vertical abnormal jaw movements (orofacial dyskinesia), ballistic movements of the contralateral forelimb (forelimb dyskinesia) and axial dystonia. We have found that inactivation of D1 dopamine receptors completely abolished orofacial dyskinesia, did not affect axial distonia and slightly reduced (aprox. 20%) forelimb dyskinesia. By contrast knock out mice lacking D2 receptors showed an increase in orofacial dyskinesia after L-DOPA treatment but axial dystonia and forelimb dyskinesia were not present. In summary, our results demonstrate that D1 and D2 receptors play a differential and complementary role in L-DOPA-induced dyskinesia and that the integrity of the D1 and D2 receptors is critical for different aspects of levodopa-induced dyskinesias. Funded by the Spanish Ministerio de Ciencia y Tecnología, ref SAF00/122 and Fundación la Caixa, Spain.

Learning and memory mechanisms of the basal ganglia: Plasticity in cortico-basal ganglia loops

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The basal ganglia have been centrally implicated in a range of cognitive and motor disorders. In normal individuals the basal ganglia may be essential to the development of behavioral routines and the kinds of relatively automatic behaviors that underlie the habits of everyday life. Still at issue, however, is what the basal ganglia do when considered as a major neural processing system of the forebrain. A key fact about the basal ganglia is that they lie as nodal points in a set of cortico-basal ganglia-thalamocortical circuits that interconnect many parts of the cortex with the basal ganglia. As the cortically directed outputs of these circuits preferentially target the frontal cortex, these basal ganglia circuits can be considered to exert a primary influence over executive areas of the cortex. In our laboratory, we have developed the hypothesis that the basal ganglia function as an adaptive mechanism to adjust cortical activity in response to detected behavioral contingencies. As a first step in examining this hypothesis, we have recorded with single electrode and multiple electrode methods in the striatum as animals learn tasks. We have found evidence for remarkable plasticity in the response properties of striatal units as animals undergo training in procedural learning tasks. Recordings in the striatum during successive bouts of learning, extinction and reacquisition indicate that the ensemble activity of striatal units can change, then can be reversed and then be reinstated. Some of these recordings have been done for identified neurons on the striatum, neurons that are thought to be local circuit interneurons. The fact that interneurons as well as projection neurons undergo such plastic changes indicates that there is a reconfiguration of network activity in the striatum during the course of learning. We have also exposed animals to dopaminergic treatments in order to identify neurons responding dynamically at the level of gene expression. Combined ensemble recording and gene-based findings should help to unravel the relative roles of the neocortex and the striatum in this process and define the mechanisms of striatal neuroplasticity.

Striatal synaptic plasticity in normal and pathological conditions

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Interactions between corticostriatal glutamatergic inputs and striatal DA receptors play a key role in striatal synaptic plasticity. LTP is induced in striatal spiny neurons both *in vitro* and *in vivo* following repetitive high-frequency stimulation (HFS) of glutamatergic corticostriatal afferents, and may represent a cellular substrate for motor learning. Striatal LTP is dependent upon activation of D1 DA receptors by endogenous DA. It is blocked by D1 receptor antagonists and is absent in animals lacking the DA and cyclic adenosine 3'-5' monophosphate-regulated phosphoprotein 32 kDa (DARPP-32). It is also blocked by a depletion of endogenous DA as produced by either 6-OHDA lesions.

The effects of L-DOPA treatment on the inducibility of LTP have thus far remained unknown. However, this form of synaptic plasticity has been hypothesized to provide a substrate for the development of abnormal motor patterns in L-DOPA-induced dyskinesia. We therefore used a corticostriatal slice preparation to measure LTP from striatal spiny neurons by intracellular recordings. We found that LTP was absent in animals sustaining unilateral 6-OHDA lesions as compared to sham-lesioned controls. We have compared the plasticity of corticostriatal synapses in two groups of hemiparkinsonian rats treated chronically with L-DOPA. One group of L-DOPA-treated animals showed motor improvement without dyskinesia, whereas the other group developed debilitating dyskinesias in response to the treatment. High-frequency stimulation of cortical afferents induced long-term potentiation (LTP) of corticostriatal synapses in both groups of animals. Intact control and non-dyskinetic, but not dyskinetic rats, showed synaptic depotentiation in response to subsequent low-frequency synaptic stimulation. The depotentiation seen in both L-DOPA-treated, non-dyskinetic rats and intact controls was prevented by activation of dopamine (DA) D1 receptors or inhibition of protein phosphatases. The striata of dyskinetic rats contained abnormally high levels of phosphoThr34-DARPP-32, an inhibitor of protein phosphatase 1. These results provide the demonstration that an abnormal information storage in corticostriatal synapses is linked with the development of L-DOPA-induced dyskinesia.

Bclx-L-mediated blockade of cell death is critical to enhance the generation of Tyrosine Hydroxylase expressing human neurons from neural stem cells

Liste I., Navarro B., Bueno C., Villa A., Martínez-Serrano A.

Oxidative stress is a well-known risk and cell-death triggering factor for dopaminergic (DA) neurons. When Tyrosine Hydroxylase (TH) is over-expressed in human neural stem cells (hNSCs), neurotrophic factors (BDNF and GDNF) and antioxidant Cu+Zn Superoxide Dismutase (SOD1cit) moderately (O_2-fold) enhanced TH expression. Anti-apoptotic Bcl-xL, however, induced a much more remarkable protection of TH + hNSCs (one order of magnitude). Bcl-xL-mediated neuro-protection is specific upon TH expression, since it does not enhance LacZ expression, and is mimicked by the pan-caspase inhibitor zVAD-fmk, confirming a block of apoptosis mechanism. Bcl-xL also enhanced (one order of magnitude) the capacity for dopaminergic differentiation of Nurr1 + immortalized lines of hNSCs and human neurosphere cultures. In fact, the production of TH + neurons by a Bcl-xL over-expressing forebrain hNSC clone and Bcl-xL over-expressing human neurospheres greatly exceed that obtained from human ventral mesencephalic tissue. Bcl-xL enhances total neuron generation by hNSCs to a lower extent, but his cannot explain the remarkable increase in TH + neurons. Rather Bcl-xL effects suggest a specific survival effect on DA neurons. From the evidence presented here obtained using three independent model systems (transgenic TH expression and normal differentiation to DA neurons, and also immortal and neurosphere cultures of hNSCs), it can be concluded that enhancing Bcl-xL expression may be of direct application for the generation of a continuous source of human TH/DA neurons. This, in turn, will facilitate the development of cell replacement and drug screening activities, in order to discover new therapeutic strategies for Parkinson's disease.

Dynamic approach of Parkinson's disease pathophysiology

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Parkinson's disease (PD) is a progressive neurodegenerative disorder which principal pathological characteristic is the loss of dopamine (DA) neurons of the substantia nigra pars compacta (SNc). Parkinsonian signs appear when dopaminergic neuronal death exceeds a critical threshold: 70-80% of striatal nerve terminals and 50-60% of SNc pericarya. After the first appearance of clinical signs, neuronal death continues and motor disturbances increase; evolution is, however, slow. This late and gradual appearance of clinical signs is due to the existence of compensatory mechanisms. Their understanding might have a clinical application in that their enhancement, if feasible, would allow to further delay the appearance of parkinsonian signs (i.e. effectively resulting in an increase of the pre-symptomatic period of Parkinson's disease) or, at least, allowing the postponement of commencing more traditional anti-parkinsonian therapy.

Unfortunately, in most of the current 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) non-human primate models, nigrostriatal lesion and the onset of PD symptoms are due to an immediate neuronal degeneration in the SNc achieved by acute injection of the toxin that prevents the pathophysiological characterization of intermediate stages such as the presymptomatic period. Recently, an animal model of PD which is able to resolve these issues has been developed (Bezard et al., 2001). In this model, repeated administration of low doses of MPTP to nonhuman primate initiates a process of neurodegeneration reminiscent of that seen in PD. The novelty of the model accrues from the fact that the protocol produces a reproducible, progressive DA cell loss over a time course of approximately one month. During the first 13-15 days of the protocol, there is significant dopaminergic loss but symptoms are not apparent. During this period, several mechanisms compensate for the increasing loss of DA, suppressing the appearance of symptoms.

Using this specific model, we thus defined the neural mechanisms, and potential biomarkers, that relate to the presymptomatic and sympomatic period of PD. Several diferent technical approaches have been used both *in vivo* and *ex vivo*. The key findings are that: (i) a role for increased DA metabolism as a mechanism of presymptomatic compensation in PD is unlikely (Bezard et al., 2001). (ii) D_2 dopamine receptor upregulation may represent a mechanism compensating for progressive dopamine loss in PD (Bezard et al., 2001) while both D_1 (Bezard et al., 2001) and D_3 receptors (Bezard et al., 2003b) either do not vary or linearly decrease, respectively. (iii) Upregulated enkephalin expression in the presymptomatic period may also represent a mechanism compensating for progressive dopamine loss in PD (Bezard et al., 2003a). (iv) Structures outside basal ganglia may compensate for progressive loss of DA in PD as shown using the 2-deoxyglucose metabolic tracing technique that has allowed the identification of changes in neuronal metabolic activity occuring before and after the appearance of parkinsonian motor abnormalities(Bezard et al., 2001).

In a pilot study, we also showed that the electrophysiological activity of the subthalamic nucleus (STN) and globus pallidus pars internalis (GPi) increased before the appearance of the symptoms suggesting that presymptomatic increases may compensate for progressive loss of dopamine in PD (Bezard et al., 2003a). Further investigating this striking issue, we recorded the GPi neuronal activity using a multichannel recording device in animals rendered parkinsonian according to the same MPTP regiment. The concomitant recording of several neurons help us in identifying not only the changes in firing frequency or firing patterns but also the modifications in the level of oscillatory and/or synchronized activity. Such an increase in synchronization is thought to be a characteristic of PD. Our preliminary results show that PD motor symptoms appear before oscillating activity of GPi neurons and its synchronization between different pallidal neurons. This suggests that synchronous oscillations in the BG network are not at the origin of PD motor symptoms and further implies that other dynamical changes have to be considered to explain the appearance of motor symptoms in PD.

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Session 3: Intracellular events and neurodegeneration in Parkinson's disease
Chairs: C. Warren Olanow and Rosario Moratalla

Mechanisms of cell death in Parkinson's disease

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At the current time the cause of Parkinson's disease remains unknown although it appears to be a complex interaction between genetic and environmental factors. In contrast, through the study of post mortem tissues and the actions of toxins such as MPTP a number of mechanisms involved in the degeneration of nigral dopaminergic cells have been uncovered. There is good but as yet inconclusive evidence that cell death in Parkinson's disease occurs through apoptosis and similarly it is possible to show that many toxins also produce nigral degeneration through apoptotic mechanisms and that interference with the apoptotic cascade can influence the survival of dopaminergic cells. Other key components of a cascade of events which occur during nigral cell death include oxidative stress, excitoxicity, nitric oxide mediated toxicity, mitochondrial dysfunction and altered protein handling. At the present time this series of events appear to be closely interrelated such that it is difficult to determine which of these may be a causative factor in nigral cell degeneration and indeed it appears likely that all are able to induce the cascade of events leading to the degeneration of dopaminergic cells.

Oxidative stress is well documented as occurring in the substantia nigra in Parkinson's disease based on alterations in levels of iron and changes in antioxidant systems. A key component appears to be the early loss of reduced glutathione which appears to be specific to substantia nigra and not to occur in other neurodegenerative diseases. Also occurring selectively in substantia nigra is a decrease in complex 1 of the mitochondrial respiratory chain. Although not present in other brain regions, a decrease in complex 1 activity may also be present in platelets, fibro blasts and in muscle. There are in addition, alterations in the activity of a key Krebs cycle enzyme, namely α-ketoglutarate dehydrogenase. The cause of mitochondrial abnormalities in Parkinson's disease remains unknown as no clear genetic abnormalities have been uncovered. However, the transfer of mitochondrial DNA from patients with Parkinson's disease to form cybrids leads to decreased complex 1 activity and oxidative stress accompanied by increased sensitivity to toxins such as MPP+ and alterations in pro- and anti-apoptotic genes.

Another key change which occurs in Parkinson's disease is the onset of reactive microgliosis leading to inflammatory changes within substantia nigra. Similarly following treatment with MPTP, gliosis involving both microglia and astrocytes is again observed. Importantly, the toxicity of MPTP to nigral dopaminergic neurones can be prevented by treatment with anti-inflammatory drugs as well as selective COX-1 and COX-2 inhibitors and it was recently shown that MPTP toxicity is enhanced in COX-2 knock out mice. Activation of glial cells

leads to increased release of toxins, including cytokines, glutamate, nitric oxide and reactive oxygen species and it is associated with a decrease in the release of trophic factors. *In vitro,* the activation of glial cells leads to dopaminergic cell death. I*n vivo,* intra-nigral administration of lipopolysaccharide (LPS) also initiates cell death as shown by a loss of tyrosine hydroxylase immunoreactivity in substantia nigra. The injection of LPS into the substantia nigra caused both reactive microgliosis and an astrocytosis associated with a marked induction of
i-NOS reactive glial cells and3 nitrotyrosine immunoreactivity showing increased nitric oxide production and toxic effects of peroxynitrite. The involvement of i-NOS in LPS induced nigral cell damage is also shown by the ability of i-NOS inhibitors to partially prevent dopaminergic cell death. Interestingly, the toxic effects of LPS to nigral dopaminergic cells is prevented not only by anti-inflammatory drugs such as dexamethasone but also by the administration of dopamine agonists, such as pramipexole. The clinical relevance of the latter finding is not clear but may contribute to the postulated neuroprotective effects of the dopamine agonists.

Oxidative damage occurs in substantia nigra in Parkinson's disease as shown by increases in markers of lipid peroxidation and DNA damage products. Importantly alterations in protein damage also occur and it appears that the substantia nigra is particularly prone to the formation of oxidised proteins which form part of its secondary anti-oxidant defence mechanisms. The formation of 4-hydroxynonenal (HNE) as a product of lipid peroxidation may be of significance in Parkinson's disease due to its highly reactive nature. HNE causes apoptotic cell death involving caspase cascades and decreases in the level of reduced glutathione and causes inhibition of complexes I and II of the mitochondrial respiratory chain. HNE also enhances cross-linking of proteins and this may be of importance to the changes in proteasomal function which occur in Parkinson's disease. In cell culture, concentrations of HNE, which by themselves are non-toxic, impair proteasomal function such that levels ubiquitinated proteins rise and this leads to both oxidative and nitrative stress. The role of alterations in the ubiquitin-proteasome system in the production of oxidative and nitrative stress is emphasised by the changes produced in cell culture by the over-expression of mutant forms of parkin and by the over- expression of a mutant form of ubiquitin which prevents the polyubiquitination of proteins and their identification for degradation by the proteasome.

There is considerable evidence for a range of biochemical changes to occur in the substantia nigra in Parkinson's disease. Each of these biochemical processes by itself may lead to a cascade of events that induces apoptotic cell death and the destruction of dopaminergic neurones. However, it is clear that a complex interaction exists between the various mechanisms which have been detected such that they appear to be heavily interrelated and this suggests that the induction of cell death in Parkinson's disease might be induced through a variety of different types of toxic insult coupled to inherent genetic susceptibility. This leads to the suggestion that there is no single cause of Parkinson's disease and indeed that Parkinson's disease may rather be a syndrome inducible through a range of different mechanisms.

Proteosomal dysfunction, protein aggregation and neuronal inclusion bodies in PD

C. Warren Olanow, K McNaught

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Parkinson's disease (PD) is an age-dependent neurological disorder characterized pathologically by preferential degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and intracytoplasmic proteinaceous inclusions known as Lewy bodies. Genetic and environmental factors are thought to contribute to the etiology of PD, and oxidative stress, excitotoxicity, mitochondrial dysfunction, and inflammation have each been implicated in the pathogenesis of cell death. Recent studies suggest that a defect in the capacity of the ubiquitin proteasome system (UPS) to clear misfolded proteins may be central to the neurodegenerative process that occurs in the different forms of PD.

Mutations have been detected in the genes encoding for the proteins α-synuclein, parkin, and ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) in a small number of familial cases of PD. Defects in α-synuclein could cause the protein to misfold and resist proteasomal clearance. Parkin and UCH-L1 are now known to be components of the UPS that act as a ubiquitin ligase and de-ubiquitinating enzymes respectively. There are reasons to consider that a defect in the UPS might also play a role in cell death in sporadic forms of PD. We have recently shown that proteasomal structure and function are impaired in the SNc in PD. This impairment in proteolysis might account for the increased levels of oxidatively damaged proteins and increased protein aggregation that are found in PD. In the laboratory, we and others have shown that proteasome inhibitors can induce selective degeneration of dopamine neurons with inclusion bodiess in both in vitro and in vivo studies. Further, proteasome inhibitors can induce a Parkinsonian syndrome in rodents with levodopa-responsive tremor, rigidity, and bradykinesia and cell loss with inclusions in the SNc, locus cerouleous, and dorsal motor nucleus of the vagus levodopa. These studies further suggest that Lewy bodies might represent defective aggresomes that form in response to accumulating levels of protein aggregates. Thus, evidence has converged to suggest that defects in the capacity of the UPS to clear abnormal proteins might account for the neuronal degeneration that occurs in both familial and sporadic cases of PD. This mechanism can account for Lewy body formation, the age-related nature of sporadic PD, and the relatively selective involvement of the SNc.

Protein misfolding and PD

Mark R. Cookson

In the rare familial forms of Parkinson's disease (PD), mutations in *alpha-synuclein* or *parkin* cause nigral cell loss and other phenotypic effects by mechanisms that are unclear. An emerging hypothesis is that subsets of neurons are vulnerable to a failure in proteasome-mediated protein turnover, which is predisposed to by these genetic factors. The presence of misfolded proteins, or unfolded proteins that have an innate tendency to oligomerize, may trigger damage in these vulnerable cells and α-synuclein has such a property and is known to induce toxicity in a variety of systems. In this talk, a number of examples of involvement of the ubiquitin-proteasome system in model cellular systems that are relevant to PD will be presented.

We have shown that expression of mutant alpha-synucleins or exposure to proteasome inhibitors both result in selective toxicity towards catecholaminergic neurons in primary midbrain cultures (Petrucelli et al., 2002, Neuron **36**, 1007-1019). Mutant alpha-synuclein also increases sensitivity to proteasome inhibitors by decreasing net proteasome function. Parkin decreases sensitivity to proteasome inhibitors and is capable of rescuing the toxic effects of mutant alpha-synuclein in primary neurons. Therefore, parkin and alpha-synuclein are linked by common effects on a specific pathway associated with selective cell death in catecholaminergic neurons. Alpha-synuclein can also affect expression of the genes involved in dopamine synthesis (Baptista et al., 2003, J Neurochem **85**, 957-968), implicating a contribution of transcriptional regulation to this process.

A third definitive gene for PD, *DJ-1*, has recently been cloned, but its relationship to α-synuclein and parkin is currently unknown. The protein appears to have a possible role in oxidative events and is a target for the ubiquitin-like modifier SUMO (Cookson, 2003, Neuron **37**, 7-10). There are two causal mutations, a large deletion that is predicted to produce an effective knockout of the gene, and a point mutation, L166P. We have recently shows that L166P destabilizes DJ-1 protein and promotes its degradation through the ubiquitin-proteasome system. Subcellular localization was broadly similar for both wild type and L166P forms of the protein. The L166P mutation therefore has the simple effect of promoting DJ-1 degradation, thereby reducing net DJ-1 protein within the cell. These observations are reminiscent of other recessive gene mutations that produce an effective loss of function and show how the ubiquitin-proteasome system handles misfolded proteins. It should be noted, however, that this explains the recessive nature of L166P DJ-1 mutation but does not necessarily support the hypothesis that the common link between different PD genes is related to proteasome function.

Session 4: Cell therapy in animal models of PD
Chair: Ann M. Graybiel

Restoring DA striatal deficiency in PD. Will it change the natural history?

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Major progress has been made in the treatment of Parkinson's disease but disease progression remains unaltered. The belief that degeneration of nigrostriatal dopamine is the major therapeutic challenge has led to considerable effort in developing cell replacement therapy aiming to restore striatal dopaminergic (DA) deficit. However, the clinical manifestations of striatal DA depletion in Parkinson's disease (i.e. rigidity, tremor, akinesia) are already well controlled with available drugs. Equally, functional surgery of the globus pallidum and subthalamic nucleus can efficiently cope with long-term motor complications associated with levodopa treatment. Thus, treatment of the motor, cardinal features, of Parkinson's disease should not be regarded as a primary therapeutic goal anymore.

The real unmet needs for the treatment of Parkinson's disease derive from the progressive and multi-systems nature of the neurodegeneration. As a result of extension of the pathological process a plethora of symptoms emerge during evolution (i.e. falls, sleepiness, fatigue, autonomic disturbance and, most importantly, cognitive abnormalities) that fail to respond to current treatment with DA agents and related drugs. It is very likely that such problems will also be resistant even to the most "successful" dopamine cellular replacement therapy. Current evidence strongly supports the notion that concentrating on restoring the nigrostriatal dopamine system should not be the ultimate goal of future research efforts in Parkinson's disease. Alternatively, research should concentrate in achieving strategies to stop disease progression and ultimately prevent its initiation.

Surgical therapies for PD: Present achievements and perspectives

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Despite significant advances in medical therapy, a large number of patients with Parkinson's disease continue have both motor and non-motor disabilities(1). For such patients, surgical treatments can be considered(2). A number of surgical procedures including making discrete targeted lesions or the application of chronic electrical stimulation to disrupt pathological activity are now being applied with a variable degree of success. To date however, no procedures or drugs are slowing down or halting the disease process. Future efforts must not only provide better symptomatic relief but also address this shortcoming. A number of interesting possibilities are emerging. The direct intraparenchymal administration of neurotrophic substances(3) or the focal application of neuroactive agents(4, 5) may be of promise. The possible application of electrical stimulation at cortical(6) and at yet to be considered non-cortical targets(7) is also intriguing. This rich pipeline will also bring gene therapy(8) and stem cell therapeutics(9). Future therapies will include molecular neurosurgery for the supply of missing and defective genes (eg DJ-1, Parkin) or the suppression of deleterious gene products (eg alpha-synuclein). Further, the newly emerging field of brain-machine interfaces may also play a role in ameliorating motor function in patients with incapacitating motor disabilities.

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Mechanisms of action of nigral grafts in experimental parkinsonism

Stephen B Dunnett

Nigral grafts can alleviate a range of motor and sensorimotor deficits in parkinsonian mice, rats, monkeys and man. However the actual mechanism of functional action is not fully understood, and understanding this question has important implications for further development of cell therapies on a rational, not just empirical, basis.

A variety of alternative mechanisms may be proposed:
i. Non-specific effects as consequences of the implantation surgery itself.
ii. Pharmacological effects, involving local secretion of missing neurotransmitter dopamine from the implanted cells.
iii. Protective effects, whereby the grafts provide support for host neurons and/or retard progression of disease degeneration.
iv. Trophic effects, whereby the graft releases local signals for host plasticity and intrinsic compensatory reorganisation or regenerative processes.
v. Reinnervation, whereby the grafted dopamine neurones reinnervate the host brain, and terminals may be under local regulation.
vi. True circuit reconstruction, whereby the grafted neurones are reintegrated into, and ultimately repair, the host neuronal circuitry.

A variety of lines of evidence to indicate that different types of implants can exhibit a functional impact through each of these mechanisms and, specifically, grafts derived from primary embryonic mesencephalon most likely exert a combination of mechanisms ii and v. Nevertheless, nigral grafts do not alleviate all deficits associated with nigrostriatal degeneration, and this is most plausibly explained by the fact that they do not achieve level vi of repair, i.e. full reconstruction of the nigrostriatal pathway. This would require long distance regeneration of embryonic axons from the grafts to distant targets which is not achieved in the adult brain. Although a number of strategies for building nigrostriatal bridges to allow such circuit reconstruction have been attempted with variable success, no fully effective strategy for full circuit repair in model parkinsonism has yet been achieved.

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Mesencephalic cell transplants in Parkinson disease: Results of double-blind trials

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The transplantation of fetal mesencephalic cells in patients with Parkinson disease was pioneered by neuroscientists in Sweden. They proceeded to develop and improve the technique over several years, and these endeavors have continued and broadened to include research on stem cells. After the initial success of fetal tissue implants in Sweden, investigators in other countries became involved in this activity. All studies were open-label investigations, and the general consensus was that this technique, although needing perfection, has shown improvement in a number of patients who had received the tissue. Since then, and in the last 2 years, two randomized, controlled, double-blind clinical trials have been reported; both sponsored by the N.I.H.

The first study (Freed et al., NEJM 2001;344:710-719) was conducted by three centers: University of Colorado, where the surgical procedure was performed; Columbia University, where recruitment and clinical evaluation of subjects was carried out; and North Shore University Medical Center, the site where FDOPA PET scans were performed. Forty patients with at least 7 years duration (mean 13.8 years) of PD were randomized to receive sham or transplant surgery using a formula weighted for age, sex, and disease duration, with pre-specified outcome analysis to evaluate subjects ≤ 60 and >60 years separately to look for an age effect. Under local anesthesia, four twist drill holes through the frontal bone were made for the needle passes into putamen. Transplant patients, but not controls, received cultured mesencephalic tissue from four embryos implanted along four needle tracts bilaterally in the putamen. No patient was immunosuppressed. The dura mater was not penetrated in the control subjects. Subjects were followed for one year with blinded evaluations. The transplant group showed significant transplant growth by ^{18}F-fluorodopa (FDOPA) PET scans and significant improvement in standardized measures of PD including UPDRS motor "off" and Schwab and England "off" scores. Placebo patients had no change in these measures. Those aged 60 or under improved while those over 60 did not. Transplant growth was the same in younger and older subjects. The primary outcome variable, a subjective Global Rating Scale, was not significantly improved in part because of a positive placebo effect in sham surgery patients. In two older transplant patients dying of causes unrelated to the transplant, surviving dopamine neurons were present in all transplant tracts. Follow-up of these subjects revealed that five subjects from the younger group developed persistent dyskinesias after prolonged withdrawal from dopaminergic therapy. Three of them underwent deep brain stimulation in the GPi to reduce these dyskinesias.

The second controlled study has not yet been published, but was presented at the Seventh International Congress of Parkinson's Disease and Movement Disorders in November 2002 (Olanow, Mov Disord 2002;17 (Suppl 5):S15). In this study, subjects were randomized into three groups: sham; implanted with mesencephalic tissue from one fetus per side; and implanted with tissue from four fetuses per side. The implants were placed only in the post-commissural putamen. Subjects were followed in a double-blind manner for 2 years. The investigators found no benefit in the transplanted subjects compared to controls, using Motor UPDRS OFF scores as the primary outcome variable. Post hoc analysis revealed benefit in milder patients (< median UPDRS of 49). About half of the transplanted patients had dyskinesias during the Practically Defined Off assessment period.

Finding a future for neural transplantation in Parkinson's disease

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In open-label trials, transplanted human embryonic dopamine neurons have been reported to ameliorate several of the motor symptoms of Parkinson's disease. Positron emission tomography studies have shown that grafted dopamine neurons can survive and reinstate dopaminergic neurotransmission. However, the outcome of two recent double-blind, placebo controlled studies have been disappointing. Not only were the effects of the tranplants at best minor, but there were also reports of graft-mediated dyskinesias in a subset of patients. The transplantation approach is further hampered by difficulties in obtaining sufficient amounts of donor tissue for each patient.

Therefore, there is clearly a need for further development of transplant technology to facilitate graft surgery and improve the chances of a positive outcome. This presentation will discuss the importance of appropriate patient selection; possible mechanisms underlying graft-related dykinesias; and the development of alternative sources of cells that can be used for transplantation. We suggest that advanced-stage Parkinson patients may be less suited for graft surgery. Concerning graft-induced dyskinesias, we propose that they are not due to a general excess of dopamine, but that small grafts innervating critical zones of the striatum are more likely to support involuntary movements. Moreover, an inflammatory response in the striatum could play a role in elicting dyskinesias. These hypotheses can be effectively tested by transplanting in a rat model of Parkinson's disease that displays L-dopa induced dyskinesias, akin to those seen in patients. Various forms of stem cells may solve the problem of limited access to suitable donor tissue. Currently dopamine neurons differentiated from human embryonic stem (ES) cells constitute one of the most compelling options. Another exciting future possibility is autografted neurons derived from the patient's own stem cells, e.g. those residing in bone marrow.

Session 5: Cell therapy and newer therapeutic strategies for Parkinson's disease
Chairs: Rosario Moratalla and Charles W. Olanow

Cell replacement and neuroprotection with stem cells

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Stem cells have been suggested to be an optimal source material for transplantation in the treatment of neurodegenerative diseases for their ability to give rise to all neural lineages and to integrate in the brain upon transplantation. One of the main candidate disorders for stem cell therapies is Parkinson's disease (PD), since most of the symptoms in that disorder are related to the progressive degeneration of a discrete population of dopaminergic neurons in the midbrain. Our work has focused on the development of two stem cell-based therapeutic strategies for PD: (1) A cell replacement strategy, based on engineering stem cells with signals that regulate the development of midbrain dopaminergic neurons. (2) A neuroprotective strategy, based on the delivery of neuroprotective signals to dopaminergic neurons.

With regard to the engineering of a dopaminergic phenotype in stem cells, in the past we have achieved a coordinated induction of midbrain dopaminergic neurons (in 80% of the cells in culture) by expressing the nuclear orphan receptor Nurr1 in neural stem cells (NSC) and exposing these cells to soluble factor(s) derived from ventral mesencephalic glial cells isolated at the time of birth of dopaminergic neurons. These results have also been confirmed with different Nurr1 expressing mouse and human stem and precursor cells. In order to identify the glial-derived signals we are performing gene chip and protein purification experiments with ventral mesencephalic glia. Likewise, we are analyzing genes regulated by Nurr1 in NSCs since Nurr1 confers NSCs responsiveness to the inductive signal/s derived from the astrocytes. Our results support a model in which the induction of a specific neuronal phenotype requires the convergence of epigenetic signals derived from neighbor cells, including glial cells, and a genetic program, including the expression of Nurr1. The identification of signaling components involved in the induction and maintenance of a dopaminergic phenotype in stem/precursor cells, will contribute to improve stem cell-based replacement strategies for PD.

In a second approach aiming at preserving the function and survival of midbrain dopaminergic neurons, we engineered NSCs to stably express different members of the glial cell-line derived neurotrophic factor family (GDNF), which are potent neurotrophic factors for midbrain dopaminergic neurons. Our results show that NSCs expressing GDNF or Persephin (PSP) integrate in the adult host striatum, survive, give rise to different cell lineages, and stably release high levels of GDNF or PSP for up to 7 months (the latest time-point tested). Moreover, in a mouse terminal 6-OHDA lesion model of Parkinson's disease, we found that GDNF- or PSP-NSCs prevented the motor deficits and the degeneration of substantia nigra dopaminergic neurons, suggesting that a neuroprotective therapy based on the delivery of GDNF or PSP by NSCs could be useful in the treatment of PD.

Carotid body cell transplantation in Parkinson's disease

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Parkinson's disease (PD) mainly results from the destruction of mesencephalic dopaminergic neurons projecting to the striatum, therefore a possible therapeutic approach to PD is the intrastriatal transplantation of dopamine-secreting cells. For the last few years we have studied the efficacy of carotid body (CB) transplants in models of PD. The CB is a bilateral organ located at the bifurcation of the carotid artery that contains electrically excitable and highly dopaminergic glomus, or type I, cells and glial-like type II cells. Glomus cells are O_2 sensitive and release dopamine (and other transmitters) in response to hypoxemia. These transmitters activate afferent sensory fibers, which synapse on brain stem respiratory neurons and produce hyperventilation[1]. Glomus cells appeared to us particularly well suited for transplantation therapy in PD as they combine a high dopamine content with good survival in hypoxic conditions. In addition, as unilateral removal of the CB has no side effects, CB autotransplantation could be used to treat PD in humans. We have shown in hemiparkinsonian rats that intrastriatal transplantation of CB cell aggregates results in almost complete recovery of the motor and sensorimotor deficits. These behavioral effects are correlated with striatal dopaminergic reinnervation and the maintenance of metabolically active CB grafts[2]. Carotid body grafts and the behavioral recovery last for almost the entire life of the animals[3]. Once we showed that unilateral CB autotransplants induce clinical and histological recovery in MPTP-monkeys[4], a pilot study was performed on six PD patients. At 1 year after transplantation, clear reductions of the blinded UPDRS III (24% to 52%) were observed in three patients; the improvement was very modest (13% and 17% reduction) in two patients, and the sole patient that showed no improvement had the most fibrosis in the CB. The age of the patient and the state of the CB tissue appear to be adversely correlated with clinical improvement following CB autotransplantation[5]. Parallel studies in rodents have indicated that the beneficial results of intrastriatal CB grafts are due to a trophic effect of the CB transplants rather than to the release of dopamine by the grafted cells[3]. We have shown that the adult CB contains large amounts of GDNF produced by glomus cells rather than by type II cells and that the ability to synthesize this trophic factor is maintained in the transplants. Therefore, GDNF and other trophic factors released by the grafted glomus cells are possibly responsible for the neuronal sprouting and nigrostriatal reinnervation observed after CB grafting. These studies indicate that glomus cells besides being dopaminergic are stable and long-lasting pumps of neurotrophic factors and, thus, could be used for cell replacement therapy in Parkinson and other neurodegenerative diseases.

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Locally-born olfactory bulb stem cells differentiate into neurons and glia in culture and upon transplantation

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At early stages of embryonic development, neuroepithelial precursor cells in the vertebrate nervous system have stem cell features, i.e., multipotentiality for neurons and glia and have the ability to self-renew.

Nonetheless, stem cells have not been described within the embryonic olfactory bulb (OB). Using tissue culture techniques, we have isolated local OB stem cells from the E14.5 mouse embryo. These cells were 99.2% nestin-positive, and proliferated extensively in culture to at least 150 cell doublings. Clonal analysis demonstrated that neurons (TuJ1+), astrocytes (GFAP+) and oligodendrocytes (O4+) could be generated from single-plated cells, indicating multipotency. At least 90% of proliferating cells expressed IGF-I, (pro)insulin, and their cognate receptors; these growth factors collaborated with FGF-2 plus EGF to promote stem cell proliferation. Whereas the IGF-I effect was additive, the actions of insulin and its unprocessed precursor, proinsulin, were synergistic with FGF-2 plus EGF. Differentiation and survival of stem cell-generated neurons and glia showed strong dependence on exogenous IGF-I, although oligodendrocyte differentiation also required insulin at low concentration. Further, the percentages of stem cell-derived neurons, astrocytes and oligodendrocytes were markedly lower in the cultures prepared from the Igf-I -/- mice when compared to those of Igf-I +/+, suggesting a specific role of endogenous IGF-I in OB stem cell differentiation. These results support the existence within the embryonic mouse OB of stem cells with specific requirements for insulin-related growth factors for proliferation or differentiation. To test the ability of OB stem cells to differentiate in vivo, cells were prepared from the OB of transgenic mice expressing green fluorescent protein (GFP) and transplanted into the adult brain. Eight weeks after transplantation, GFP-positive cells having neuronal and glial morphologies were found in the striatum, corpus callosum, and cortex suggesting that OB stem cells differentiated into neurons and glia. The use of the OB as a source of stem cells for transplantation in animal models of Parkinson´s disease is currently being investigated.

Mesencephalic cell transplants for PD: Lessons for future developments

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The adult mammalian brain is a regenerative system capable of incorporating embryonic stem (ES), progenitor or fetal primary neurons into functional circuitries. These implanted neurons and glia grow physiologically and functionally to specifically repair damaged or degenerated neuronal connections, as shown in a multitude of animal models. New therapeutic non-pharmacological methodology involves cell and synaptic renewal or replacement in the living brain to restore function of neuronal systems, including the dopaminergic (DA) system in Parkinson's disease (PD). Understanding the cell biological principles for generating functional DA neurons in lieu of the diseased can provide many new avenues for better treatment of patients with PD. Recent laboratory work has focused on using stem cells as a starting point for exogenous or endogenous derivation of the optimal DA cells for repair. Using fetal DA cell therapy in PD patients and stem cell derived DA neurons in animal models, it has been demonstrated that functional motor deficits associated with PD can be reduced after application of this new technology. Evidence shows that the underlying disease process does not destroy the transplanted fetal DA cells, although the patient's original DA system degeneration progresses. The optimal DA cell regeneration system would reconstitute a normal network capable of restoring feedback-controlled release of DA in the nigro-striatal system. The success of cell therapy for neurological diseases is limited by access to preparation and development of highly specialized dopaminergic neurons found in the A9 and A10 region of the substantia nigra (SN) in the ventral mesencephalon, as well as technical and surgical steps associated with transplantation.

Alternatives to donor human fetal cells are now studied as more practical cell sources for future clinical treatments; for example, xenogeneic fetal cells, stem cells, progenitor cells and genetically modified cells. These cells are studied functionally and in terms of developmental and transcription factors such as Nurr 1, PitX 3, SHH and markers relating to growth cone behaviors and cell type specification from ES to dopamine neuronal types.

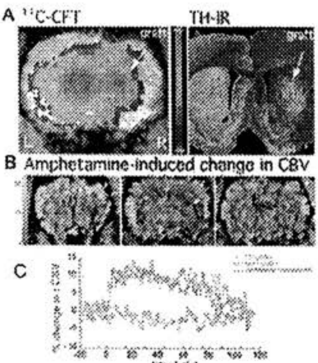

Fig. 1. (*A*) By using PET and the specific DAT ligand [11C]CFT, we identified specific binding in the right grafted striatum, as shown in this brain slice (*A, Left*) acquired 26 min after injection of the ligand into the tail vein (acquisition time was 15 sec). Color-coded (activity) PET images were overlaid with MRI images for anatomical localization. The increase in [11C]CFT binding in the right striatum was correlated with the postmortem presence of TH immunoreactive (IR) neurons in the graft (*A, Right*). (*B*) Neuronal activation mediated by DA release in response to amphetamine (2 mgykg) was restored in animals receiving ES grafts. Colorcoded maps of the percentage of change in rCBV are shown at two striatal levels for control (*Upper*) and an ES cell-derived DA graft (*Lower*). A 6-OHDA lesion results in a complete absence of CBV response to amphetamine on striatum and cortex ipsilateral to the lesion (*Upper*). Recovery of signal change in motor and somatosensory cortex (arrows) and to a minor extent in the striatum was observed only in ES-grafted animals. (*C*) Graphic representation of signal changes over time in the same animal shown in *B*. The response on the grafted (red line) and normal (blue line) striata was similar in magnitude and time course, whereas no changes were observed in sham-grafted animals (green line). Baseline was collected for 10 min before and 10 min after monocrystalline iron oxide nanocolloid injection, and amphetamine was injected at time 0. cc, corpus callosum. (L.M. Bjorklund et al., Proc. Natl. Acad. Sci. 99 2344-2349, 2002).

At the cellular and molecular level of brain repair, we investigated specific axon guidance factors in the adult brain. By transplanting ES and fetal neuroblasts into various locations in the brain of animal models, we determined that most neuroanatomical systems can reconnect and create reparative interactions. In animal models, implanted fetal or ES cell derived dopamine neurons can survive long-term and gradually reduce signs of PD. Therefore, the current state of the art cell therapy for PD appears to require transfer of appropriate and selectively placed DA neurons in patients that are responsive to DA substitution therapy. Possibly, the emergence of stem cell generated DA neurons in concert with improved surgical and technical approaches may provide a more optimal intervention for some patients with PD.

Cellular and gene therapy delivery in animal models of Parkinson's disease and Huntington's disease

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Cell replacement and gene therapy are two promising areas for treatment movement disorders. In this regard, glial cell-derived neurotrophic factor (GDNF) potently supports the viability and phenotypic expression of dopaminergic nigral neurons. The consistent success of GDNF in animal models of Parkinson's disease (PD) has led to a clinical trial that was subsequently abandoned due to minimal efficacy, significant side effects, and post-mortem evidence that this intraventricular GDNF fails to penetrate the brain parenchyma sufficiently to access vulnerable nigrostriatal neurons. We have performed a series of studies in which the GDNF gene was delivered directly to the nigrostriatal system of young, aged and MPTP-treated monkeys using a lentiviral vector system. Robust and consistent gene expression (2.5-3.5 μg/mg protein of GDNF) for up to 8 months was seen in all animals. Anterograde and retrograde transport of secreted GDNF was noted. Injections of lenti-GDNF to aged monkeys increased the number, size, and THmRNA expression within nigral neurons and dopamine and TH-immunoreactivity within the striatum. Enhanced fluordopa uptake was also seen on PET scan. Injections of lenti-GDNF to MPTP-treated monkeys reversed the motor deficits seen on a clinical rating scale and an operant hand-reach task. The loss of fluorodopa uptake seen on PET was prevented by lenti-GDNF. The loss and shrinkage of nigral neurons, as well as the loss of nigral THmRNA and striatal dopamine was also prevented by lenti-GDNF. Minimal toxicity was observed following lentivirus injections. These data support the concept that lentiviral delivery of GDNF may prevent the structural and functional consequences seen in patients with PD. Interestingly, administration of recombinant GDNF also has been demonstrated to be effective in rodent models of Huntington's disease. Huntington's disease (HD) is an autosomal dominant disorder caused by an expanded polyglutamine (CAG) tract at the IT15 locus on chromosome 4. These excessive repeats lead to the degeneration of striatal and cortical neurons resulting in a devastating cognitive, psychiatric, and motor disorder for which no treatments are available. Neurotrophic factors support the viability of striatal neurons suggesting that they might prevent the inevitable neural degeneration and its accompanying functional decline associated with HD. We investigated whether glial cell line-derived neurotrophic factor (GDNF) delivered by an adeno-associated virus could provide structural and functional neuroprotection in a rat model of HD. Lewis rats received bilateral injections of either AAV-GDNF (n=12) or AAV-green fluorescence protein (AAV-GFP, n=12) into the striatum followed two weeks later by chronic subcutaneous infusions of the mitochondrial toxin, 3-nitropropionic acid (3-NP, 38 mg/kg). All rats underwent 4 weeks of behavioral testing and were then sacrificed. Following 3-NP, the performance by AAV-GFP treated rats on a raised platform motor task deteriorated while performance by AAV-GDNF treated rats near normal ($p<0.001$). AAV-GDNF treated rats also received better scores on a blinded semi-quantitative neurological scale compared to rats receiving AAV-GFP ($p<0.001$). Histological analyses supported our behavioral findings. 3-NP treated rats receiving AAV-GDNF displayed 70% more NeuN-immunoreactive (ir) neurons compared to 3-NP treated rats receiving AAV-GFP ($p=0.002$). Similar findings were seen with dopamine-and-adenosine-3'5'-monophosphate-regulated phosphoprotein (DARPP-32) staining. These data indicate that the viral-mediated gene transfer of GDNF into the striatum provides neuroanatomical and behavioral protection in a rodent model of HD. The urgency to find a clinical intervention for this disease has lead to a surge in research focusing on the transplantation of human neural stem cells (hNSC). hNSC are attractive candidates for cell replacement therapy because they are capable of self renewal and can be maintained in culture for long periods of time prior to transplantation. Here we show that hNSC survive transplantation into the rat striatum, migrate extensively, differentiate into neurons and astrocytes and attenuate the behavioral deficits seen in a rat model of Huntington's disease.

POSTERS

Functional uncoupling of CB1 cannabinoid receptors in the caudate-putamen of mu opioid knockout mice

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Although the existence of functional links between the endogenous cannabinoid and opioid systems has been already been demonstrated in numerous studies, extensive research is still needed to elucidate the biochemical mechanisms involved in this interaction. The absence of m-opioid receptors has recently been shown to abolish THC conditioned place preference in mice, suggesting that m-opioid receptors activity can modulate the pathways involved in the rewarding effects of cannabinoids.

To test the possible existence of changes in the expression and/or functional activity of cannabinoid receptors in m-opioid receptors knockout mice, we have performed quantitative receptor autoradiography of CB1 cannabinoid receptors and activation of GTP-binding proteins by CB1 agonists in the brains of wild-type and homozygous knockout mice. No significant differences were obtained in the levels of CB1 receptors in the brains of m-opioid receptors mutant mice. In contrast, the activation of CB1 receptors by the cannabinoid agonist WIN 55,212-2 was dramatically reduced in the caudate-putamen of m knockout animals when compared to wild-type controls. Since co-expression of CB1 receptors and m-opioid receptors in the same patch neurons of the rat caudate-putamen nucleus has recently been reported, the present results suggest that deletion of m-opioid receptors uncouples CB1 receptors located in these striatal neurons.

Neuroprotective effect of VIP in a mouse model of Parkinson´s disease by blocking microglia activation

Mario Delgado and Doina Ganea

Parkinson's disease (PD) is a common neurodegenerative disorder with no effective protective treatment, characterized by a massive degeneration of dopaminergic neurons in the substantia nigra (SNpc) and the subsequent loss of their projecting nerve fibers in the striatum. To elucidate PD pathogenic factors, and thus to develop therapeutic strategies, a murine PD model based on the administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used extensively. It has been demonstrated that activated microglia cells actively participate in the pathogenesis of MPTP-induced PD through the release of cytotoxic factors. Because current treatments for PD are not effective, considerable research focused lately on a number of regulatory molecules termed microglia-deactivating factors. Vasoactive intestinal peptide (VIP), a neuropeptide with a potent anti-inflammatory effect, has been found to be protective in several inflammatory disorders. This study investigates the putative protective effect of VIP in the MPTP model for PD. VIP treatment significantly decreases MPTP-induced dopaminergic neuronal loss in SNpc and nigrostriatal nerve-fiber loss. VIP prevents MPTP-induced activation of microglia in SNpc and striatum and the expression of the cytotoxic mediators, iNOS, interleukin 1b, and numor necrosis factor a. VIP emerges as a potential valuable neuroprotective agent for the treatment of pathologic conditions in the central nervous system, such as PD, where inflammation-induced neurodegeneration occurs.

Dual control of basal ganglia output by means of the thalamic parafascicular nucleus

JL Lanciego, Gonzalo N, Castle M, Vázquez A, Aymerich M, Obeso JA

This work is focused on the study of neuronal circuits arising from the rodent caudal intralaminar nuclei and their presumed role on basal ganglia function. Emphasis was placed on the analysis of the architecture of thalamostriatal and thalamo-subthalamic projections in albino rats. Our major interest was to elucidate whether thalamic inputs were related to projection neurons or local circuit neurons within targeted structures (striatum and subthalamic nucleus). Projections coming from the parafascicular nucleus to the striatum displayed a patchy organization throughout the matrix compartment. These patches are composed by dense terminal axonal arborizations, often containing striatal neurons projecting either to the entopeduncular nucleus (medial globus pallidus in primates) or to the external globus pallidus, as well as neurons projecting to the substantia nigra reticulata. The thalamostriatal projections under scrutiny were also seen to be in register with all the major classes of striatal interneurons (nitrergic neurons, neurons containing the calcium binding protein parvalbumin, and cholinergic interneurons). Subthalamic neurons projecting to either the entopeduncular nucleus or to the external globus pallidus are the presumed postsynaptic target for fibers coming from the sensorimotor part (dorsolateral) of the parafascicular nucleus. In summary, glutamatergic axons arising from the parafascicular nucleus might exert a dual control of the striatal output, either by directly exciting striatal projection neurons or indirectly by means of a previous synaptic contact onto a striatal interneuron which in turn modulates the activity of projection neurons. Furthermore, thalamic inputs can also gain access to basal ganglia output nuclei via subthalamo-pallidal projecting neurons, neurons receiving glutamatergic thalamo-subthalamic projections. Thus, activation of either circuit has an opposite physiological effect on the basal ganglia output nucleus. Taken together, these data suggest that the parafascicular nucleus may influence neuronal activity in the direct and indirect circuits and could be considered as an additional component of the basal ganglia motor loops.

Bclx-L-mediated blockade of cell death is critical to enhance the generation of Tyrosine Hydroxylase expressing human neurons from neural stem cells

Liste I., Navarro B., Bueno C., Villa A., Martínez-Serrano A.

Oxidative stress is a well-known risk and cell-death triggering factor for dopaminergic (DA) neurons. When Tyrosine Hydroxylase (TH) is over-expressed in human neural stem cells (hNSCs), neurotrophic factors (BDNF and GDNF) and antioxidant Cu+Zn Superoxide Dismutase (SOD1cit) moderately (CO_2-fold) enhanced TH expression. Anti-apoptotic Bcl-XL, however, induced a much more remarkable protection of TH + hNSCs (one order of magnitude). Bcl-XL-mediated neuro-protection is specific upon TH expression, since it does not enhance LacZ expression, and is mimicked by the pan-caspase inhibitor zVAD-fmk, confirming a block of apoptosis mechanism. Bcl-XL also enhanced (1-2 orders of magnitude) the capacity for dopaminergic differentiation of Nurr1 + immortalized lines of hNSCs and human neurosphere cultures. In fact, the production of TH + neurons by a Bcl-XL over-expressing forebrain hNSC clone and Bcl-XL over-expressing human neurospheres greatly exceed that obtained from human ventral mesencephalic tissue. Bcl-XL enhances total neuron generation by hNSCs to a lower extent, but his cannot explain the remarkable increase in TH + neurons. Rather Bcl-XL effects suggest a specific survival effect on DA neurons. From the evidence presented here obtained using three independent model systems (transgenic TH expression and normal differentiation to DA neurons, and also immortal and neurosphere cultures of hNSCs), it can be concluded that enhancing Bcl-XL expression may be of direct application for the generation of a continuous source of human TH/DA neurons. This, in turn, will facilitate the development of cell replacement and drug screening activities, in order to discover new therapeutic strategies for Parkinson's disease.

Melatonin attenuates oxidative stress induced during preparation of primary dopamine neurons for grafting

D.M. Marchionini, C.E. Sortwell, M.F. Fleming and T.J. Collier

Transplantation of embryonic ventral mesencephalic (VM) tissue aims to replace dopamine in the striatum that is lost in Parkinson's disease (PD).

Unfortunately graft viability is poor, therefore strategies must augment graft survival before transplantation can be a practical therapy for PD. Oxidative stress is hypothesized to be a threat to graft viability but it has been unclear which aspect of the grafting procedure may be associated with this risk. Here we demonstrate that the dissociation process generates oxidative stress and that addition of the anti-oxidant melatonin to the dissociation medium attenuates this stress. We report that dissociated VMs compared to whole VMs have more protein oxidation, as measured by reactive carbonyl derivatives in a Western blot. 10μl VM microislands were plated on poly-D-lysine coated plates with hormone supplemented serum free medium for 5 days. The addition of 100 or 250 μM melatonin during the dissociation process yielded a significant increase in the number of tyrosine hydroxylase immunoreactive neurons; 175 ± 6.9 and 218 ± 9.0% of control, respectively. We have previously shown that systemic melatonin administration to the host significantly enhances graft-induced recovery of amphetamine-induced rotational behavior. Ongoing studies will compare graft survival and functional recovery in animals that receive cells untreated or supplemented with 250 μM melatonin during the dissociation procedure. We show that the dissociation process alone triggers oxidative stress, and utilization of anti-oxidants at this point in the grafting paradigm may augment graft survival.

Transgenic mice over-expressing G6PD in nigrostriatal neurons: General characteristics and resistance to MPTP toxicity

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Idiopathic Parkinson's disease (PD) is a multifactorial disorder of unknown origin characterized by degeneration of nigrostriatal neurons. A possible pathogenic factor is the oxidative stress inherent to dopamine metabolism. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the synthesis of the NADPH, has been shown to be essential to reduce glutathione and to protect cells against oxidative stress (1-3). So, we have developed a tissue-specific transgenic mouse (TG) overexpressing G6PD. The transgene expression was directed to the substantia nigra using a rat 9 kb tyrosine hydroxylase (TH) promoter (4).

The expression pattern of G6PD was analyzed by *in situ* hybridization and immunohistochemistry. Expression was mainly observed in ventral mesencephalic neurons, although appreciable staining was also seen in hippocampus, hypothalamus, olfactory bulb, locus coeruleous, amygdala, and cortex. Besides in the CNS, high levels of G6PD were also found in adrenal medulla. G6PD expression in carotid body or sympathetic ganglia was, however, below our level of detection. An increase in G6PD enzymatic activity was detected in substantia nigra and striatum of TG mice compared with the littermate controls. We have studied whether over-expression of G6PD protects dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neuro-toxicity. MPTP (40 mg/kg) was injected subcutaneously to groups of 5-7 littermate controls and 5-7 TG animals and they were sacrificed seven days later to examine the nigrostriatal pathway. Although detailed quantitative studies are still underway, observations from three separate experiments suggest that the effect of injection of MPTP on nigrostriatal neurons is reduced in TG animals. This is observed not only in the number of TH(+) nigra cells, but more importantly, in the level of striatal innervation. These data suggest that metabolic alterations could underlie the pathogenesis of PD and that stimulation of NADPH production could be a potential therapeutic approach to this disease.

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Nitric oxide triggers the toxicity due to glutathione depletion in midbrain cultures through 12-lipoxygenase

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Glutathione (GSH) depletion is the earliest biochemical alteration shown to date in brains of Parkinson's disease (PD) patients. However, data from animal models show that GSH depletion by itself is not sufficient to induce nigral degeneration. We have previously shown that non-toxic inhibition of GSH synthesis with L-buthionine-(S,R)-sulfoximine (BSO) in primary midbrain cultures, transforms a nitric oxide (NO) neurotrophic effect, selective for DA neurons, into a toxic effect with participation of guanylate cyclase (GC) and cGMP-dependent protein kinase (PKG). Here we demonstrate that arachidonic acid (AA) metabolism through the 12-lipoxygenase (12-LOX) pathway is also central for this GSH-NO interaction. LOX inhibitors (NDGA and baicalein) but not cyclooxygenase (indomethacin) or epoxygenase (clotrimazole) ones, prevent cell death in the culture, even when added 10h after NO treatment. Furthermore, AA addition to GSH depleted cultures precipitates a cell death process that is indistinguishable from that initiated by NO, in its morphology, time course and 12-LOX, GC and PKG dependency. The first AA metabolite through 12-LOX enzyme, 12-HPETE, induces cell death in the culture and its toxicity is greatly enhanced by GSH depletion. In addition we show that if GSH synthesis inhibition persists for up to 4 days without any additional treatment, it will induce a cell death process that also depends on 12-LOX, GC and PKG activation. In this study, therefore we show that the signalling pathway AA/12-LOX/12-HPETE/GC/PKG may be important in several pathologies in which GSH decrease has been documented, like PD. The potentiating effect of NO over such signalling pathway, may be of relevance as part of the cascade of events leading to and sustaining nerve cell death.

Abnormal CaMKII function mediates synaptic and motor deficits in experimental parkinsonism

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The NMDA receptor complex represents a molecular key element in the pathogenesis of long-term synaptic and motor abnormalities in Parkinson's Disease (PD). Here we show that NMDA-NR1 subunit and PSD-95 protein levels are selectively reduced in the striatum of dopamine (DA) denervated rats. These effects are accompanied by an increase in striatal levels of alpha $Ca2+$/Calmodulin-dependent protein kinase II (alpha CaMKII) autophosphorylation. While normalizing alpha CaMKII autophosphorylation levels, the intrastriatal administration of the CaMKII inhibitor KN-93 is able to reverse both the alterations in corticostriatal synaptic plasticity and the deficits in spontaneous motor behaviour that are found in this animal model of PD. The same beneficial effects are produced by a regimen of L-DOPA treatment that is able to reduce alpha CaMKII autophosphorylation. These data indicate that an abnormal autophosphorylation of alpha CaMKII plays a causal role in the alterations of striatal plasticity and motor behaviour that follow DA denervation. Normalization of CaMKII activity may be an important underlying mechanism of the therapeutic action of L-DOPA in PD.

Wnt-1 signalling in the development of ventral midbrain dopaminergic neurons

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The Wnt family of secreted protein are involved in cellular proliferation and differentiation during development and deletion of the Wnt-1 gene results in loss of midbrain and hindbrain structures. We have recently found that Wnt-1 regulates proliferation of ventral midbrain neural precursors. We now show that Wnt-1 promotes cell cycle regulation by upregulating cyclins D1 and D3 and downregulating p27 and p57 mRNAs. By blocking GSK-3b, we are able to mimic the effect of Wnt-1 on the number of TH+ cell/field, indicating that Wnt-1 signals through the canonical b-catenin pathway.

Zinc release in the substantia nigra and Glutamate and GABA neurotransmission

Manuel Rodríguez Díaz, Teofilo Jorge Alonso, Juan Perdomo Díaz, Tomás González Hernández, Rafael Castro Fuentes, Pablo Varela, Sergio Gonzalez and José García Dopico

There is existing evidence which shows a transmitter role for zinc in different telencephalic centres and which suggests that it is involved in neurodegenerative disorders of forebrain neurones. In the present study, the release of zinc was studied with microdialysis methods in the substantia nigra (SN) of rats, a mesencephalic centre involved in the pathophysiology of Parkinson´s diease. Evidence was found for an extracellular pool of zinc that increases after depolarization with high doses of K^+ or glutamate-receptor agonists. Low doses of zinc increased extracellular glutamate (GLU) and decreased glutamine, suggesting an inhibitory role of zinc on nigral GLU-transporters. Low doses of GLU increased extracellular zinc, suggesting a facilitatory action between GLU and zinc release. Previous evidence suggests that both GLU and zinc are involved in the degeneration of telencephalic cells. Present data suggest a cooperative action of zinc and GLU in increasing the vulnerability of SN-cells, which could be involved in the pathophysiology of Parkinson´s diease.

Neuronal activity of the subthalamic nucleus during active, passive and reflex movements in Parkinson's disease

M.C.Rodriguez-Oroz; F.Alonso-Frech; M.Alegre; J.Guridi; J.Artieda; I.Zamarbide; M.Rodriguez-Diaz; J.A.Obeso

The subthalamic nucleus is a key structure in normal motor control and in the pathophysiology of PD. We performed extracellular single cell recording in the subthalamic nucleus(STN) during surgery in 15 patients. Modifications of neuronal activity in relation with active (ballistic) and passive movements of the wrist and in response to tendon jerk tapping of the arm and knee were assessed in 33 well isolated neurones. The electromyographic (EMG) activity and joint position of the limb during the manouvres were recorded simultaneously. Twenty two neurons were related to active ballistic movements. Most units (77,6 %) showed an increase of activity 60-115 ms before EMG onset or joint displacement. A few (9.2%) showed a period (100-200 ms long) of decreased activity coinciding with EMG onset followed by a rebound increment. Eighteen neurones responded to passive movement increasing the discharge rate (up 200 %) some 70-130 msec after the movement. Six neurons responded to tapping (phasic streching) with a brief (60 ms) but very reproducible increment in firing with a latency of 67-78 ms. All these neurones were located in the dorsolateral region of the nucleus. We conclude that the STN in PD is involved in both movement initiation and stretch reflex mechanisms. The different patterns of activity encountered suggests that the STN sensorimotor activity is related with movement modulation.

Neuronal differentiation of primate parthenogenetic embryonic stem (ES) cells Cyno-1 after transplantation into the rat striatum

R. Sánchez Pernaute, L. Studer, O. Cooper, & O. Isacson

We have previously reported that mouse ES cells differentiate *in vivo* into neurons (Deacon et al., 1998). Neuronal differentiation, and, in particular, differentiation into dopamine neurons, increased when ES cells were grafted at a low concentration decreasing cell-to-cell interaction (Bjorklund et al., 2002). Here we investigated the *in vivo* differentiation of Cyno-1 primate ES cells (Cibelli et al., 2002). These cells are parthenogenetic (developed from an unfertilized egg) but develop normally *in vitro* and *in vivo*, giving rise to cells from the three germ layers. Dopamine neurons are obtained *in vitro* when cells are grown in appropriate conditions (Cibelli et al., 2002). To determine the potential of Cyno-1 for transplantation in Parkinson's disease models, we examined survival, proliferation and differentiation of Cyno-1 cells grafted into the rat striatum. Early differentiation was investigated in naïve rats at 2 weeks.

Cyno-1 cells in the host brain formed tubular neuroepithelial structures, resembling the neuroepithelial rosettes observed in culture dishes. Many cells expressed stage specific embryonic antigen 4 (SSEA-4) at this stage, but neurons expressing ßtubulin were already present in high numbers at the periphery of the neuroepithelial structures. These neurons co-expressed a primate specific neuronal marker. Tyrosine hydroxylase (TH) expression was not detected at this early stage. Next we grafted undifferentiated Cyno-1 ES cells at low cell density into 6-OHDA-lesioned rats and examined the outcome 3-4 months post transplantation. As observed for rodent ES, undifferentiated cells differentiated *in vivo* predominantly into neurons but in contrast to mouse ES cells, the majority of these ES cell-derived neurons adopted a forebrain (BF-1 positive) phenotype. TH positive neurons were observed but, in contrast to rodent ES cells, noradrenergic neurons were more abundant than dopaminergic ones. These studies demonstrate that undifferentiated Cyno-1 cells give rise to neurons *in vivo* but the dopaminergic fate does not appear to be favored. Together with the presence of cells derived from other germ cell layers in the grafts, our results underscore the need for instructed differentiation of primate ES cells and selection of appropriate neuronal populations for cell replacement therapy.

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Increased levels of TGF-beta-1 in striatal regions of PD patients may contribute to the neurodegenerative process

Amelia Sánchez-Capelo and Jacques Mallet

Increased levels of Transforming growth factor β-1 (TGF-β-1) have been found in the central nervous system of patients with Parkinson's disease (PD), Alzheimer's disease, Down's syndrome and ischemic lesions. In PD patients, TGF-β-1 are three times higher in the striatal regions (caudate nucleus and putamen) than in normal individuals. The effect on the mesoestriatal dopaminergic system of this increment is still unknown. In vitro studies on embryonic dopaminergic cells have produced opposite results, from neuroprotection to neurotoxicity. We have observed that striatal overexpression of TGF-β-1, by recombinant adenovirus TGF-β-1 gene transfer, in 6-OHDA dopamine-depleted striata decreases the survival and functionality of transplanted embryonic dopaminergic neurones derived from the ventral mesencephalon (Sánchez-Capelo et al., 1999). Moreover, TGF-β-1 overexpression in the mesostriatal system of an MPTP mouse model of PD decreased the number of mesencephalic dopaminergic neurones compare to MPTP-treated mice. This effect also involved more extensive DA depletion in the striatum and decrease in dopaminergic transmission to the postsynaptic target, measured by striatal mRNA expresion of preproenkephalin A. In the absence of MPTP, TGF-β-1 greatly decreased the number of dopaminergic neurones in the ventral mesencephalon of fully mature mice. These results show that an increase in TGF-β-1 levels aggravate the parkinsonian status of MPTP mice. TGF-β-1 receptors type I (ALK1, ALK2, and ALK5), and type II (T-β-R-II) are expressed in the substantia nigra and striatum of normal and parkinsonian mice. In an wide variety of systems TGF-β-1 is a strong inducer of apoptosis. The increment of TGF-β-1 may therefore be a risk factor for the development of PD. I will present these results and discuss possible mechanisms of neurotoxicity induced by TGF-β-1 in PD.

The intranigral injection of thrombin induces *in vivo* selective degeneration of dopaminergic neurons along with the activation of microglia

Tomás-Camardiel M., Carreño-Müller E., Herrera A.J., de Pablos R.M., Venero J.L., Cano J. and Machado A.

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The most significant pathological features of PD are the presence of oxidative stress (Dexter et al., 1994; Jenner and Olanow, 1998) and immune/inflammatory activity shown as a dramatic proliferation of reactive amoeboid macrophages and microglia in the substantia nigra (SN) (McGeer et al., 1988a,b; Hirsch et al., 1998).

A great amount of works pointed out the significant importance of the inflammatory process and the activation of microglia in the induction of degeneration of dopaminergic system of the nigral system. We have studied the effect of one physiological compound, thrombin, on the possible inflammatory reaction and its effect on the neurons of the nigrostriatal system. Thrombin is a multifunctional serine protease, best known for its role in the blood coagulation cascade. It is derived from its zymogen, prothrombin, and converts fibrinogen into fibrin, activates platelets, and stimulates the proliferation of vascular smooth muscle cells (Davey and Luscher, 1967; Davie et al., 1991; McNamara et al., 1993).

Seven days after the injection of different concentrations of thrombin into the nigrostriatal pathway, it was observed a strong macrophage/microglial reaction in SN, pointed out by immunostaining using OX-42 and OX-6 antibodies and by the induction of iNOS, Il-1beta; and TNF-alpha. The SN was far more sensitive than the striatum to the inflammatory stimulus induced by thrombin injection. Moreover, a selective damage to dopaminergic neurons was produced after thrombin injection, evidenced by the loss of tyrosine hydroxylase mRNA-expressing cells bodies and the unaltered transcription of glutamic acid decarboxylase mRNA in SN and striatum. These thrombin effects could be produced through its capability to induce the activation of microglia described in *in vitro* studies, and are in agreement with the effects described for other proinflammatory compounds. Thrombin effects are produced by its biological activity since they almost disappeared when thrombin was heat-inactivated or injected along with its inhibitor alpha-NAPAP. These results could have special importance in some degenerative processes of the nigrostriatal dopaminergic system and could be also involved in some kind of Parkinson's disease.

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