

Interactions Between MPTP-Induced and Age-Related Neuronal Death in a Murine Model of Parkinson's Disease

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ABSTRACT: Abiotrophy is hypothesized to explain the onset and time course of deficits in Parkinson's disease (PD). Abiotrophy includes: 1) exposure to agent(s) causing the death of dopaminergic nigrostriatal neurons (DNSns), 2) gradual death of DNSns with age, 3) summation of 1) and 2) until DNSn numbers fall below a threshold for detectable neurological deficits. Murine DNSn death following methyl-phenyl-tetrahydropyridine (MPTP) exposure occurs according to an exponential relationship while age-related death of DNSns occurs according to a second exponential relationship. Summing the two exponential losses overestimates experimental DNSn death showing a simple abiotrophic model is not sufficient. Aged murine DNSns greatly increase their dopamine synthesis and the density of their striatal axon terminals which may explain the above threshold. Murine DNSns die gradually after MPTP exposure and L-deprenyl treatment rescues MPTP-damaged DNSns by a previously undiscovered action, altering the abiotrophic interactions and possibly explaining the slowed progression of PD found with deprenyl treatment.

RÉSUMÉ: Interactions entre la mort neuronale induite par le MPTP et celle qui est reliée à l'âge dans le modèle murin de la maladie de Parkinson. L'abiotrophie est une hypothèse qui a été émise pour expliquer le début et l'évolution des déficits dans la maladie de Parkinson (MP). L'abiotrophie inclut: 1) l'exposition à un (des) agent(s) causant la mort des neurones dopaminergiques nigrostriés (nsDNS); 2) la mort graduelle des nsDNS avec l'âge; 3) l'addition de 1) et 2) jusqu'à ce que le nombre des nsDNS s'abaisse sous un seuil où les déficits neurologiques deviennent détectables. La mort des nsDNS chez le rat exposé au MPTP survient selon une relation exponentielle, alors que la mort des nsDNS reliée à l'âge survient selon une autre relation exponentielle. L'addition de ces deux pertes exponentielles surestime la mort expérimentale des nsDNS, démontrant qu'un simple modèle abiotrophique n'est pas suffisant. Les nsDNS du rat âgé augmentent considérablement leur synthèse de dopamine et la densité des terminaisons de leurs axones striataux, ce qui peut expliquer le seuil ci-haut mentionné. Les nsDNS du rat meurent graduellement après exposition au MPTP et le traitement par le L-déprényl préserve les nsDNS endommagés par le MPTP par un mécanisme jusqu'ici inconnu, altérant les interactions abiotrophiques et expliquant possiblement le ralentissement de la progression de la MP observé avec le traitement par le déprényl.

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The death of dopaminergic nigrostriatal neurons (DNSns) is the cardinal pathological feature of PD¹ and results in a reduction of the dopaminergic innervation of striatal neurons and depleted striatal dopamine (DA) concentrations.² Yet the cause of DNSns death in PD remains obscure. Various causes have been proposed including viruses,³ toxic exposure⁴ or abnormal immune mechanisms.^{5,6} Much of the support for an environmental toxin as a causative agent has accrued from the finding that exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine methyl-phenyl-tetrahydropyridine (MPTP) can cause a Parkinson's-like syndrome in humans and experimental animals.⁷

It has also been considered that aging of DNSns might contribute to the genesis of PD (see⁸ for a more complete consider-

ation). Human DNSns appear to die with an almost linear rate of 4-7% per decade.^{9,10} Parkinson's brains at autopsy retain less than 27% of the number of DNSns found in adolescence¹¹ so that a loss of DNSns due to age-related death sufficient to produce clinically evident PD would only be found in persons living to 100 years or beyond. Hence the normal rate of DNSn age-related death would not be sufficient to produce the losses of DNSns found in patients with the disease since its usual onset is before 70 years of age. PD might then result from an acceleration of the mechanisms causing age-related death of DNSns. Although we know little of the mechanisms of age-related neuronal death in mammals (see¹² for a review), it seems unlikely that PD is simply a consequence of an increase in a normative mechanism.⁸

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Components of an Abirotrophic Model

The concept of abirotrophy first proposed by Gower^{8,13} offers another framework within which to examine neuronal death in PD. The basic components of abirotrophy as applied to neurodegenerative diseases are schematized in Figure 1 and can be summarized as follows:

Component 1 — that a variety of external agents including toxins can cause the death of specific populations of neurons; Component 2 — that neurons die gradually during life as part of the aging process; Component 3 — that some threshold loss of neurons subserving a particular behavioural function is required before neurological deficits are detectable; Component 4 — that in cases in which the neuronal death caused by an external agent is insufficient to exceed the threshold, the gradual addition of age-related death can cause the threshold to be exceeded some years after the exposure to the external agent so that the delay between exposure and the onset of detectable neurological deficits will be inversely proportional to the magnitude of the neuronal death caused by the external agent, to the rate of age-related death and to the magnitude of the threshold.

As applied to PD, the concept of abirotrophy has gained indirect support from positron emission tomography (PET) studies of normal asymptomatic persons at a variety of ages¹⁴ and of

MPTP-treated monkeys.¹⁵ Normal persons showed a correlation between the ages of the individuals and the uptake of [¹⁸F]-6-fluoro-L-dopa from the blood into the striatum which was taken to indicate an almost linear reduction in the number of dopaminergic nerve endings in the striatum with aging (there was a 53.3% decrease in uptake over an age range of 22 to 80 years). The monkeys were treated with unilateral injections of MPP+ (MPTP's active metabolite) into one carotid artery so that one striatum was exposed to the toxin while the other was not exposed. There was markedly reduced striatal positron emission after [¹⁸F]-6-fluoro-L-dopa administration compared to the uninjected side but the animals remained asymptomatic. Together the PET studies support two of the concepts of the abirotrophic model: firstly, that age-related loss of the terminals of DNSns occurs continuously and almost linearly across the human lifespan and secondly that a significant proportion of the DNSn terminals can be lost without producing detectable neurological deficits in primates i.e. without exceeding some threshold loss.

Yet the validity of abirotrophy as a framework for understanding the time course of PD has not been directly examined in an invasive model for the disease. We have studied both the MPTP-induced death and the age-related death of DNSns in an isogenic

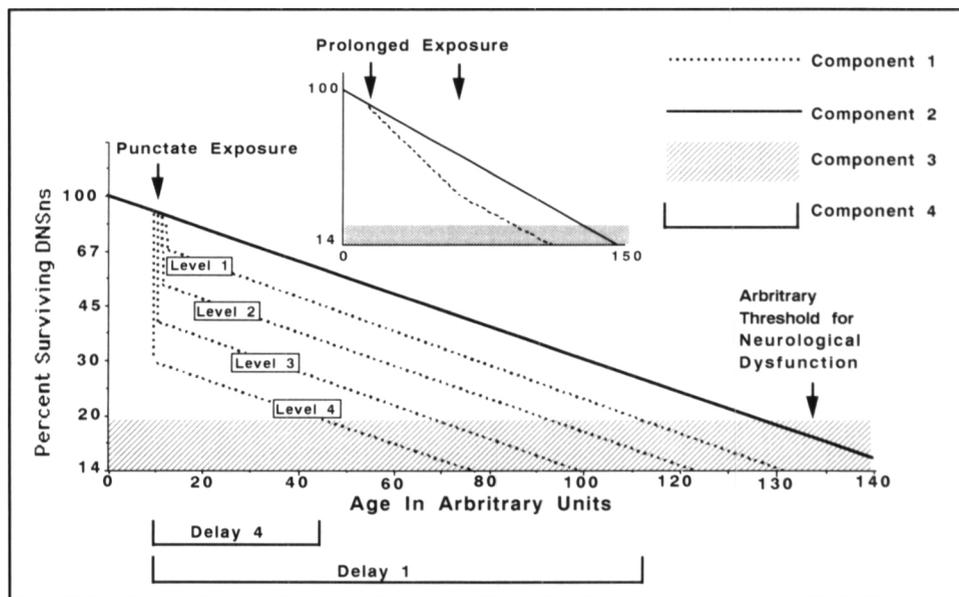


Figure 1 — The four basic components of abirotrophy as applied to PD. The ordinate shows the predicted survival of dopaminergic nigrostriatal neurons (DNSns; expressed as % survival and presented on a logarithmic scale) and the abscissa, age, expressed in arbitrary units. Component 1 demonstrates that the acute exposure to a variety of external agents can cause the rapid death of DNSns and that the extent of death depends upon the level of exposure to the external agent. Component 2 shows the age-related death of DNSns occurring naturally as part of the aging process, with continual loss of DNSns throughout the lifespan according to an exponential relationship which is linearized by the logarithmic ordinate. As depicted for component 3, some threshold loss of DNSns subserving a particular behavioural function must be exceeded before neurological deficits are detectable. In the case of PD, based on neurochemical measurements, it has been proposed that as little as 20% of the DNS population can support behavioural function with no evidence of neurological deficits. Component 4 shows that in cases in which the neuronal death caused by the external agent is insufficient to exceed the threshold, the gradual addition of age-related death can cause the threshold to be exceeded some years after the exposure to the external agent so that the delay between exposure and the onset of detectable neurological deficits will be inversely proportional to the magnitude of the neuronal death caused by the external agent, the rate of age-related death and the threshold level. The inset depicts the same relationships between aging and external agent exposure, but now illustrates this relationship for chronic exposure to the external agent.

mouse strain. Together MPTP-induced death and age-related death of DNSNs provide all of the components necessary to study an abiotrophic model of PD. The isogenic mice offer the advantage that their average life span is about 102 weeks so DNSNs can be studied using interventional techniques over their entire life which is not practical in primates. In this manuscript, we will review some of our recent findings in the context of abiotropy and present some new findings relative to the validity of abiotrophic concepts and the modulation of the interactions between toxically-induced and age-related neuronal death.

REVIEW OF RECENT RESEARCH PERTINENT TO ABIOTROPHY AND PARKINSON'S DISEASE

Actions of MPTP on Catecholaminergic Neurons – An example of Component 1

Early reports of MPTP's actions proposed that the toxin selectively destroyed DNSNs at low dosages in primates and thereby produced a PD-like syndrome.^{16,17} This selective destruction was proposed to be based on the high affinity of the toxin for neuromelanin which served to concentrate MPTP in the somata of DNSNs¹⁸ and induce their destruction by oxide radicals.¹⁹ Since monoamine oxidase B (MAO-B) inhibitors were found to block MPTP's destruction of DNSNs when co-administered with the toxin while MAO-A inhibitors failed to

offer protection,^{20,21} it was proposed that MAO-B inhibition prevented conversion of MPTP to a toxic metabolite in the brain and that this conversion was under the action of MAO-B. Initially, it was proposed that the conversion occurred within DNSNs.²²

Rodent DNSNs appeared to be relatively resistant to the toxin since mice and rats did not show sustained decreases in striatal dopamine levels or behavioural deficits with dosages that were ten fold or more higher than those required to produce striatal dopamine depletion and deficits in primates.^{23,24} The sensitivity of primates to lower doses was proposed to be related to higher concentrations of MAO-B and neuromelanin in the primate brain as compared to that of the rodent. Yet that proposal is no longer tenable since it has been shown that MAO-B is not located in dopaminergic neurons in either rodents²⁵ or primates.²⁶⁻²⁸ Rather, MAO-B is found primarily in astroglia and in serotonergic neurons and a few histaminergic neurons. Hence the conversion of MPTP to its toxic metabolite, MPP⁺, does not occur within DNSNs but rather within astroglia.²⁹⁻³¹

The different dosage requirements of primates and rodents appear to depend on differences in biodegradation and excretion of MPTP and its metabolites rather than neuronal susceptibility.³²⁻³⁶ MPTP and MPP⁺ remain within the primate brain for 6-7 days after a single systemic treatment while in rodent brains most of the toxin and its active radical are cleared within 48

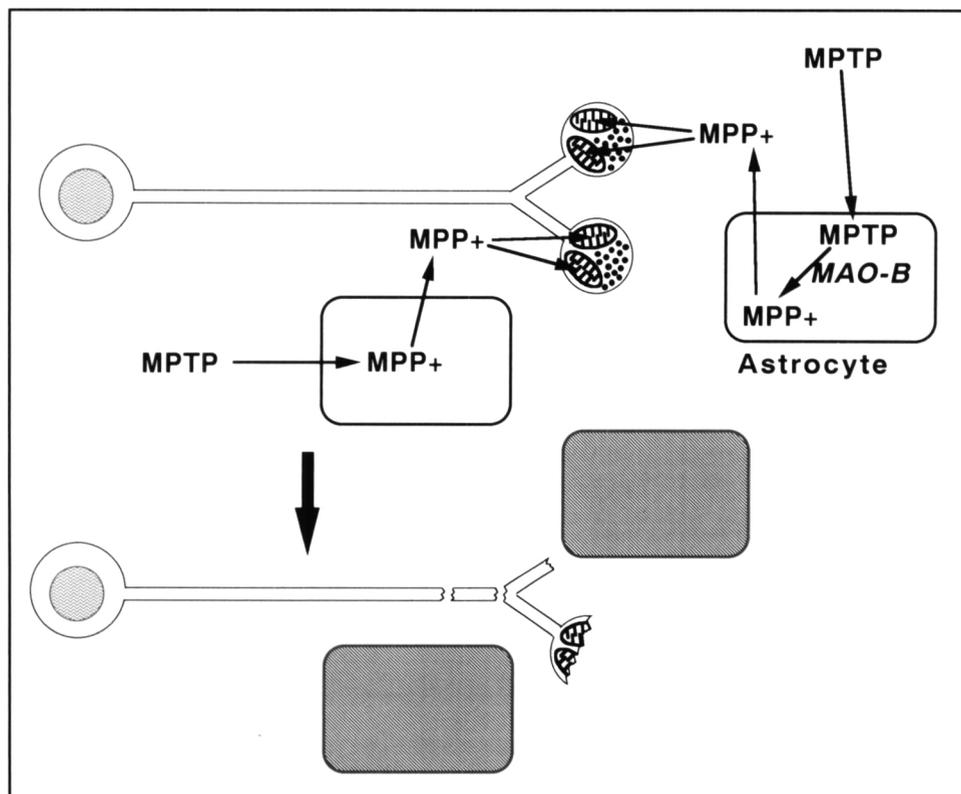


Figure 2 — Postulated biodegradation and neurotoxic mechanism of MPTP. In order for MPTP to exert a neurotoxic effect, it must be first metabolized to its toxic metabolite MPP⁺. This conversion requires MAO-B and takes place in astroglial cells where MAO-B is predominantly localized in the central nervous system. The MPP⁺ is subsequently released into the extracellular space where it is taken up into catecholaminergic terminals and is then bound to the NADH complexes of mitochondria and blocks the production of ATP resulting in fragmentation of the terminal axons of catecholaminergic neurons.

hours of a systemic dose. Lastly, the action of the toxin is not limited to DNSNs in either species. A variety of catecholaminergic neuronal subtypes are damaged or lost in both primates and rodents.³⁷⁻⁴⁰

More recent research has markedly altered our understanding of the cellular action of MPTP on catecholaminergic neurons (see ⁴⁰ for more details). Figure 2 summarizes those understandings. MPTP must be converted to its active radical MPP⁺ in order to initiate its toxic action. The conversion takes place in astroglial cells²⁹⁻³¹ and the MPP⁺ is subsequently released into the extracellular space where it is transported into catecholaminergic terminals by specific uptake systems in the axon terminal membranes.⁴¹ MPP⁺ is then bound to the NADH complexes of mitochondria⁴²⁻⁴⁴ and blocks the production of ATP^{45,46} resulting in fragmentation of the terminal axons of catecholaminergic neurons.⁴⁷ Hence the major action of MPTP on catecholaminergic neurons like DNSNs is similar to that of a distal axonal lesion, an observation which was initially made by Kitt and her coworkers.⁴⁸

Different neuronal phenotypes differ greatly in their capacity to survive axonal lesions.⁴⁹ Their ability to initiate the protein synthetic changes necessary for axonal repair appears to be related to the extent and the time course of death in a given neuronal population. We believe that the intrinsic capacity of different neuronal phenotypes to respond to and survive axonal lesions explains the apparent differences in the responses of different catecholaminergic neurons to MPTP.⁵⁰ Some neurons suffering an axonal lesion characteristically show a decrease in the synthesis of transmitter-related enzymes and an increase in the synthesis of cytoskeletal proteins like tubulin.^{51,53} For example, catecholaminergic neurons can show a loss of immunoreactivity for tyrosine hydroxylase (TH) immediately following exposure to the toxin.⁴⁸ The loss of TH immunoreactivity in catecholaminergic neurons correlates with a decrease in catecholamine content and synthesis in their target tissues.⁴⁰ We have found that murine DNSNs either recover their TH immunoreactivity within 20 days of their last exposure to MPTP or they die.⁵⁴ In contrast, dopaminergic retinal amacrine neurons do not recover TH immunoreactivity for 6 to 8 months after MPTP exposure but all of the amacrine survive.⁵⁰ The amacrine show the same initial decrease in TH synthesis as DNSNs but do not die in response to the MPTP damage.

Hence DNSNs appear to "decide" whether to live or die during the first 20 days after MPTP exposure.⁵⁵ We have found that the numbers of surviving neurons gradually decrease over the first 10-15 days after MPTP exposure reaching a steady level by 20 days (schematized in Figure 3). Increasing the dosage of the toxin produced an increasing loss of DNSNs according to an exponential relationship ($\% \text{ surviving DNSNs} = e^{-.004(\text{dose})+4.4}$). Interestingly, the mice show a dose-dependent reduction in locomotor activity after MPTP which recovered by 15-20 days after exposure to the toxin⁵⁶ and which is correlated with a recovery of striatal dopamine content toward normal levels with an almost identical time course.⁵⁴ Both the recovery of locomotor activity and the recovery of striatal dopamine synthesis occurred concurrently with the gradually progressive death of DNSNs (see Figure 3). We have recently shown that both recoveries are paralleled by increased dopamine synthesis and a recovery of TH immunoreactivity in the DNSNs that "decide" to survive the toxin (unpublished observations).

Age-Related Death of DNSNs — Component 2

We have determined the time course of age-related death of DNSNs and three other monoaminergic phenotypes over the average life-span of the C57BL mouse. Whole nuclear or retinal counts were made for immunocytochemically-identified neurons at multiple time points between 8 and 102 weeks of age and the data plotted with curve fitting using a non-linear estimation technique.⁵⁷ The best fitting relationships were found to be represented by exponential equations of the form $\% \text{ surviving neurons} = 100 e^{-(\text{rate constant} \times \text{age in weeks}) + \text{constant}}$. The rate constants for the four monoaminergic phenotypes ranged from 0.001 to 0.016 with DNSNs having a rate constant of 0.013. Hence the gradual contribution of age-related death to an abiotrophic relationship would differ for each phenotype. The greatest absolute number of DNSNs are lost early in life and a constant proportion of the DNSNs surviving at any age are lost every week of life (so that 1.2% are lost between 8 and 9 weeks of age and 0.3% are lost between weeks 99 and 100). The ordinate is scaled logarithmically in Figure 1 in order to plot the exponentially decreasing percentage of surviving DNSNs as a linear relationship.

The loss of the immunocytochemically-identified DNSNs could have represented either the death of the neurons or a loss of TH immunoreactivity in a proportion of the neurons due to a failure of TH synthesis or a change in its immune structure. Hence, we carried out experiments in which the retrograde axonal tracing agent Fluoro-Gold (FG) was micro-injected into one striatum and after allowing three days for retrograde transport back to the somata of origin, the ipsilateral substantia nigra was serially sectioned and examined for TH immunocytochemistry.⁵⁸ Multiple fields within the extent of the substantia nigra were examined with both fluorescence microscopy to detect those neuronal somata which contained FG (FG+) and bright-field microscopy to determine the percentage of FG+ somata which were TH immunopositive (TH+). Substantia nigra were

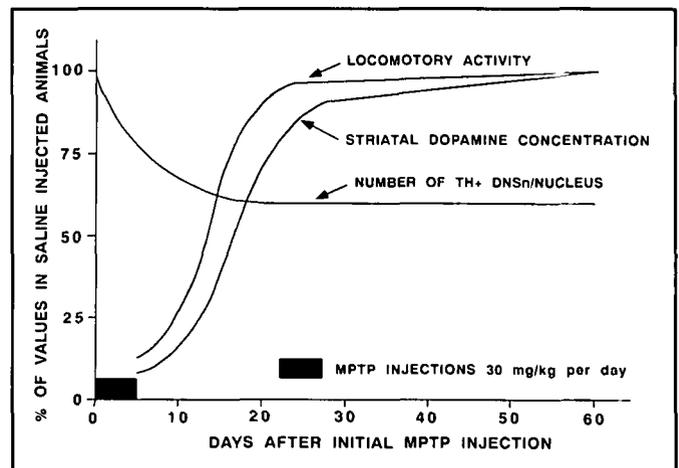


Figure 3 — Relationships amongst behavioral recovery, striatal dopamine concentrations and the survival of DNSNs following a cumulative dose of 150 mg/kg MPTP delivered in divided doses over five days. The relationships are schematized from data reported in.⁵⁶ Note that behavioral recovery and the recovery of striatal dopamine concentrations are correlated and occur while the death of DNSNs is still in progress.

examined at both 8 weeks and 104 weeks of age. It is known that about 96% of substantia nigra neurons sending axons to the ipsilateral striatum are dopaminergic and TH+.^{59,60} If the loss of DNSNs with aging was due to a loss of TH immunoreactivity rather than neuronal death then the percentage of FG+ neurons that were TH+ in the 104 week old animals would be decreased below the percentage found at 8 weeks of age. We found that 96.3 ± 4.8 were FG+ and TH+ at 8 weeks of age and 96.0 ± 4.8 were FG+ and TH+ at 104 weeks of age. This convincingly demonstrated that the loss of TH+ DNSNs across the lifespan of the C57BL mice was due to progressive neuronal death.

Modulation of the Rate of DNSNs Age-Related Death by Early Life MPTP Exposure – Component 1 and Component 2 Do Not Sum by Simple Algebra

One of the basic questions arising from application of the abiotrophic model to PD is: Does the rate of age-related neuronal death remain unchanged after the loss of a proportion of the neurons due to exposure to a toxic agent? If the rate remained unchanged as schematized in Figure 1, the rate of loss with aging in the exposed animals would parallel that for the unexposed animals. If the rate is accelerated by the toxic exposure then the time between the exposure and the onset of detectable clinical deficits (i.e. the age at which the percentage of surviving neurons falls below the threshold value) would be shortened relative to those in Figure 1. Alternately, deceleration of the rate of age-related death due to the toxic exposure would lengthen the time before onset of detectable clinical deficits.

We treated 8-week-old mice with either 60 mg/kg or 150 mg/kg MPTP in divided doses over five days.⁵⁵ TH+ immunocytochemistry was done for alternate serial sections through the entire substantia nigra at 81 weeks of age for the 60 mg/kg treated animals and at 37 weeks of age for the 150 mg/kg treated animals. The variances determined for the age-related death at the two ages were summed with those for the dose relations determined for the 60 mg/kg and 150 mg/kg MPTP doses in 8-week-old animals to obtain the predicted values for the percentage of surviving neurons if the rate of age-related death was unchanged after the MPTP exposure as illustrated in Figure 1. The predicted values were $25.3 \pm 1.3\%$ (mean + sd) and $30.9 \pm 1.8\%$ for the 60 mg/kg dose examined at 81 weeks and the 150 mg/kg dose examined at 37 weeks of age respectively. The experimental values for the treated animals were found to be $32.7 \pm 2.7\%$ for the 60 mg/kg dose examined at 81 weeks and 39.8 ± 1.7 for the 150 mg/kg dose examined at 37 weeks. The predicted and experimental values are significantly different ($p < .001$) demonstrating that early life exposure to the toxin produced an apparent net slowing of the rate of age-related death in those DNSNs surviving the toxin.

This result indicates that abiotrophy cannot necessarily be applied to the Parkinson's model according to the simple addition used in Figure 1. Rather, it appears that the interval between the toxic exposure and the onset of detectable neurological deficits would be longer than that expected if the net rate of age-related death of the DNSNs remained unchanged. Although at first examination it seems that early life MPTP exposure may alter the cellular mechanisms which determine the rate of age-related death another alternative must be considered. That is, the toxin may selectively destroy those DNSNs that are "pro-

grammed" for age-related death early in life (i.e. the same DNSNs are preferentially vulnerable to MPTP-induced death and age-related death), so that if our study was continued beyond 81 weeks of age we might find that there was a return to the normal rate of age-related death. At present it is not known whether the same neurons are susceptible to aging and to toxin exposure; if dual susceptibility turns out to be the case, it would require major changes to our current posits pertaining to abiotrophy.

Compensatory Increases in Dopamine Synthesis in DNSNs Surviving Age-Related Death – A Possible Basis for Component 3

We have found that an average DNSn surviving age-related death shows a progressive increase in the dopamine content of its striatal terminal axons⁶¹ so that striatal dopamine concentrations do not decrease as rapidly as DNSNs numbers with aging. More recently we have used two different agents which block dopamine synthesis or degradation to show that dopamine synthesis is increased in aged neurons.⁵⁸ For example, the numbers of DNSNs decrease from $3,294 \pm 261$ to 1048 ± 131 per nucleus between 8 weeks and 104 weeks of age in the C57BL mice. Over the same period dopamine synthesis per average DNSn increases 2.7 fold so that total striatal dopamine synthesis remains almost constant, maintaining striatal dopamine concentrations with aging. This increased synthesis and content might allow the surviving DNSNs to compensate for the age-related loss of their fellow neurons and thereby would be major factor in determining the threshold level for neurological deficits. The compensation would not only require increased dopamine synthesis but would also require the reinnervation of striatal neurons that had lost DNSn innervation due to the age-related death.

METHODS FOR CURRENT RESEARCH

C57BL/6NNia mice were treated with 20 mg/kg of MPTP or saline intraperitoneally for each of five consecutive days. Beginning on the 8th day, the animals were treated with deprenyl (0.25 mg/kg or 10.0 mg/kg) or saline every second or third day (see schedule in the results). On the 25th day, the mice were anaesthetized with pentobarbital, perfused with 4% paraformaldehyde in phosphate buffer and the dissected brains immersed in 20% sucrose. The brains were bisected longitudinally along the midline and the half brains from saline and deprenyl treated animals were glued together using Tissue-Tek so that the surface landmarks were in longitudinal register. The glued brains were frozen in -70 degree C. methylbutane and then 10 μ m serial sections were cut through the SNc. Alternate sections were reacted with a polyclonal antibody for tyrosine hydroxylase (TH, Eugene Tech.), then incubated with biotinylated secondary antibody, followed by incubation with HRP conjugated avidin and finally reacted with diaminobenzidine and H_2O_2 . TH immunoreactive somata containing nuclear profiles were counted from the alternate sections taken serially through entire SNc nuclei in the deprenyl and saline halves and the values were corrected for counting from the alternate 10 μ m sections.⁶² Intervening sections were Nissl stained to define nuclear outlines. In a second series of experiments pairs of brains for 8-

week-old and 81-week-old animals were prepared similarly and serial sections were taken through the striatum (with the exception that a two stage perfusion was used with the second at pH 6.8 to enhance the immunoreactivity of TH+ terminals). The striatal sections were immunoreacted for TH as above.

RESULTS OF CURRENT RESEARCH PERTINENT TO ABIOTROPHY AND PARKINSON'S DISEASE

Compensatory Striatal Axon Sprouting in DNSNs Surviving Age-Related Neuronal Death is Likely a Factor in Determining Component 3

We have attempted to determine whether the increased dopamine content and synthesis described above for the aging murine striatum reflects increased content/synthesis per axon terminal or alternately increased numbers of axon terminals consequent to axonal sprouting by the surviving DNSNs. We immunoreacted serial sections through pairs of 8 week and 104 week striatum for TH as described above. The pairs of sections

were examined under interference contrast microscopy and randomly chosen striatal fields were digitized using a frame grabber system and a 386 computer (see⁵⁰ for details). The percentage area of in focus TH+ immunoreacted tissue was measured for randomly chosen 100 micron × 100 micron areas within the striatal fields. The values for the 8 week and 104 week animals were normalized against the mean immunoreactive area determined for the 8 week animals. Each value was divided by the mean number of DNSNs that had previously been found for the two ages.^{57,58} The distributions obtained for the multiple measurements are shown in Figure 4. Each distribution presents values for striatum that were immunoreacted as pairs so as only to compare striatal tissue that was exposed to immunoreactants at identical concentrations and for identical durations.

If the immunoreacted area measurements can be taken as an estimate of dopaminergic terminal axon length per surviving DNSn (i.e. that the terminals only increase in length and not in average diameter) then the distributions indicate that an average DNSn at 104 weeks of age supports a total terminal axonal arborization that is 1.9 to 2.5 times as great as that for a similar DNSn at 8 weeks of age. This increase in axonal extent indicates that the increases in striatal dopamine content and synthesis in the striatal terminals of DNSNs in aging mice largely reflects compensatory sprouting by the surviving neurons.

We believe that the increases in dopamine content and synthesis and the sprouting of the terminal axons of DNSNs serve to compensate for the age-related death of a proportion of the neurons and may form the basis for the threshold loss necessary for the evolution of neurological deficits. That is, the sprouting and increased dopamine synthesis would reflect the establishment of new connections between surviving DNSNs and striatal neurons which had lost their nigral input due to age-related death of other DNSNs. The sprouting and increased synthesis could serve to compensate for the loss of DNSNs and therefore form the basis of the deficit threshold. The threshold (i.e. the loss value at which neurological deficits first appeared) would represent the value at which sprouting and increased dopamine synthesis are unable to maintain sufficient dopaminergic transmission to provide for normal function. This view is of course hypothetical until it is demonstrated that the sprouted terminal axons form working synapses with denervated striatal neurons. Yet differences in the capacity for compensatory sprouting in elderly humans might be an important factor in determining those individuals who show age-related neurological deficits at an early age. Alternately, those individuals whose neurons are able to sprout actively and compensate for a higher percentage loss of their fellow neurons might retain intact neurological function well beyond the normal age.

Deprenyl Reduces the Death of DNSNs Independently of its Blockade of the Conversion of MPTP to MPP+ – Modulation of Component 1 by a Pharmacological Agent

The DATATOP project^{63,64} and an independent study^{65,66} have reported that deprenyl (Selegiline) increases the interval between the onset of detectable neurological deficits in PD and the progression of those deficits to the point where L-Dopa therapy is required. The relationship between the abiotrophic model for neuronal death and the actions of deprenyl in slowing the progress of the disease are unclear. First, deprenyl might slow the rate of age-related death thereby altering the slope of the

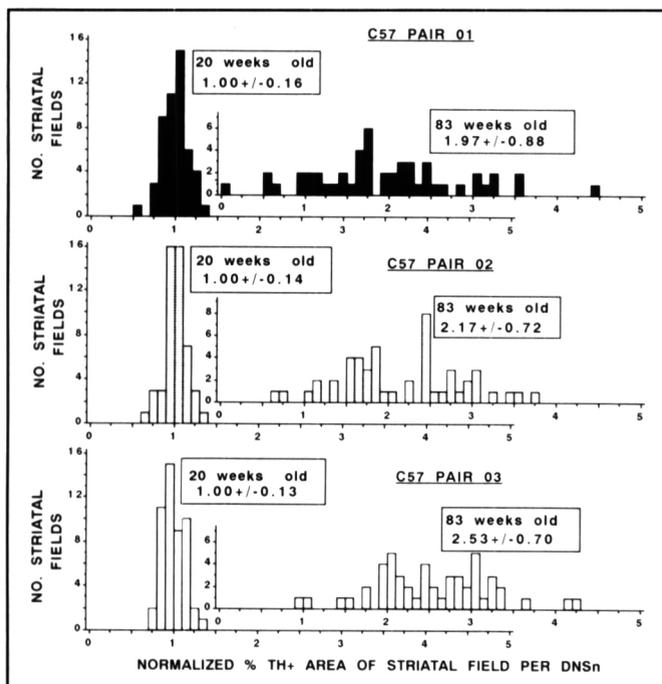


Figure 4 — Dopaminergic nigrostriatal (DNS) axon terminal density in young and aged C57BL mice. The percentage area of in focus TH+ immunoreacted tissue was measured for randomly chosen 100 × 100 micron areas within the striatal fields of young adult and aged mice. The normalized % TH+ area within the striatal fields was determined independently for each pair of glued brains; the values from the 8-week-old mouse and the 104-week-old mouse were divided by the mean immunoreactive area determined for the 8 week animal. Each value was subsequently divided by the mean number of DNS neurons that had been found for the two ages in order to obtain the presented values. The plots represent the distributions obtained for the multiple measurements of striatal fields from three separate pairs of young and aged mice. In all three cases, the distribution for the 81-week-old mouse is presented immediately adjacent to the distribution observed in the 8-week-old mouse that formed the glued pair. Note that in all three aged animals, the terminal density per surviving dopaminergic nigrostriatal neuron was 1.9 - 2.5 fold higher than that found in the paired 8-week-old mouse.

portion of the summed loss relationship of DNSns which is below the threshold for neurological deficits. Alternately, the death caused by a toxin like MPTP, might sum with age-related death in a continuous manner (see the inset in Figure 1) and deprenyl might reduce the ongoing neuronal damage and death caused by the toxin.⁶⁷ Finally, deprenyl might serve to modify the threshold relationship by increasing the compensatory capacity of the surviving neurons i.e. increase their capacity to synthesize dopamine.⁶⁸

We have carried out an experiment designed to determine whether deprenyl can rescue neurons that are dying after MPTP damage. We treated mice with intraperitoneal saline or 30 mg/kg of MPTP for five consecutive days (a cumulative dose of 150 mg/kg of MPTP). Our previous work had shown that a cumulative dose of 150 mg/kg results in the death of $42.4 \pm 8\%$ of DNSns when administered at 8 weeks of age.⁴⁰ Previous studies had co-administered deprenyl with MPTP in order to block the astroglial conversion of MPTP to MPP⁺ (see Figure 5). Therefore those previous experiments had prevented the DNSns from being damaged by MPP⁺.

Given our data that DNSns die gradually over a 20 day period after MPTP exposure, we delayed treatment with deprenyl until 72 hours after the fifth MPTP dose. In mice, the metabolism and excretion of MPTP is markedly accelerated compared to primates^{22,23,34} and the action of deprenyl can be

investigated after the conversion of MPTP to MPP⁺ is completed.⁶⁹ All of the MPTP is converted to MPP⁺ within 4 hours of an injection in mice. Hence, as illustrated in Figure 5 deprenyl would not be able to block the conversion of MPTP to MPP⁺ when administered 72 hours after the last dose. Rather, as described above the terminal axonal damage produced by the daily doses over five days would have been completed prior to the administration of deprenyl⁴⁷ and the DNSns would have entered into the "decisional" phase relative to survival after the MPTP exposure. Deprenyl or saline was administered to the mice on days 8, 10, 12, 15, 17, 19, 22 and 24 after the first MPTP dose and the animals were sacrificed on day 25. Two doses of deprenyl were used, 0.25 mg/kg and 10 mg/kg. Drawing from studies in rats,⁷⁰⁻⁷² 10 mg/kg is sufficient to almost completely block MAO-B activity and a proportion of MAO-A activity as well. Approximately 40-60% of MAO-B activity would be inhibited by the 0.25 mg/kg dose.

We found that animals treated with saline alone had 3014 ± 304 DNSns (mean \pm SEM) while those treated with MPTP followed by saline had 1872 ± 187 (38% loss). The animals treated with MPTP followed by deprenyl had 2535 ± 169 (16% loss) for the 0.25 mg/kg dose and 2586 ± 161 (14% loss) for the 10 mg/kg dose. Hence, even when delayed by 72 hours, deprenyl treatment rescued 61% of the neurons that "decided" to die when treated with saline after MPTP.

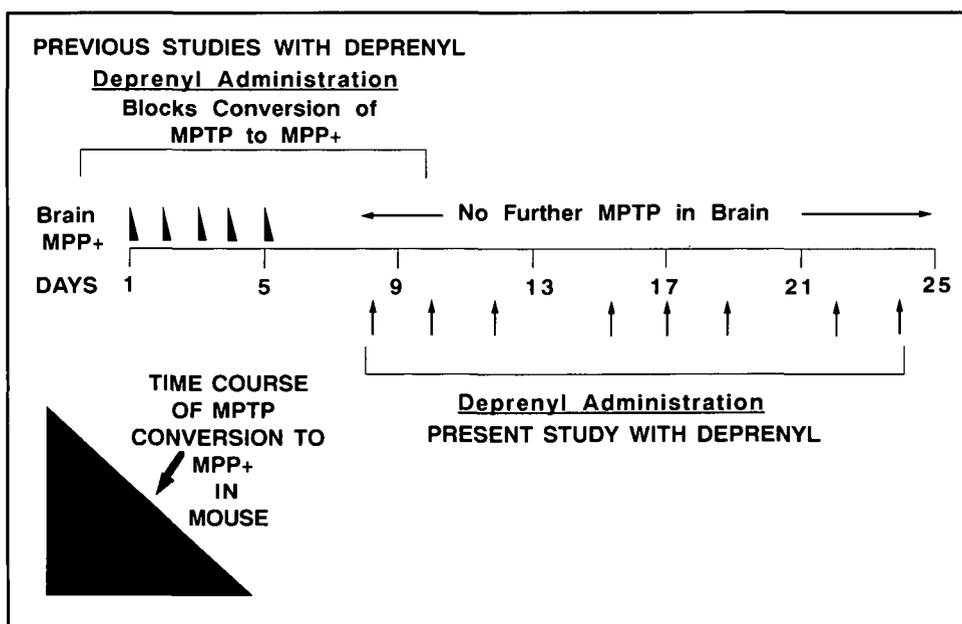


Figure 5 — Schemata of experimental design for study examining the effect of deprenyl administration on survival of dopaminergic nigrostriatal neurons following MPTP exposure. In the present study, 8-week-old C56BL mice were treated with MPTP (30 mg/kg/day, intraperitoneally) for 5 consecutive days. The time course for the biodegradation and excretion of MPTP described by others is detected by the solid triangles. Note that MPTP is rapidly cleared from mice such that all MPTP and MPP⁺ has disappeared from the brain by 24 - 48 hours after toxin administration. Deprenyl (0.25 or 10 mg/kg) or saline was administered to the mice on days 8, 10, 12, 15, 17, 19, 22 and 24 after the first MPTP dose (depicted by the arrows) and the animals were sacrificed on day 25. Important to this design is the fact that deprenyl treatment commenced after all MPTP had been cleared from the body in order to determine if deprenyl has a mechanism of action which is independent of its ability to inhibit the conversion of MPTP to MPP⁺. This design is in marked contrast to that used in previous studies in which deprenyl was co-administered with MPTP.

DISCUSSION OF CURRENT RESEARCH

If the data showing compensatory sprouting of the striatal terminals of DNSNs in aged mice can be applied to the aging human striatum then measurements of striatal dopamine of [¹⁸F]-6-fluoro-L-dopa uptake studies cannot be directly interpreted in terms of numbers of surviving DNSNs. For example, the decrease of 53.3% in [¹⁸F]-6-fluoro-L-dopa striatal uptake between persons at 20 and 82 years of age¹⁴ would not indicate a similar loss of DNSNs. Rather the compensatory sprouting with aging would cause the difference in [¹⁸F]-6-fluoro-L-dopa striatal uptake to greatly overestimate the percentage difference in surviving DNSNs at the two ages. Similarly, Hornykiewicz and his coworkers⁷³ have used striatal dopamine measurements from post mortem parkinsonian brains to determine the decrease in striatal dopamine concentration that corresponds to the threshold for the onset of detectable neurological deficits in the disease and have proposed a value of about 20%. They have estimated that striatal dopamine concentrations must fall to that level before deficits can be detected. If aging human DNSNs are capable of compensated dopamine synthesis and axonal growth similar to that which we have found for the aged mouse neurons then the threshold in terms of surviving DNSNs would be considerably lower than 20%.

Our research is the first to show that deprenyl can rescue toxically-damaged neurons from death by a mechanism other than the blockade of the conversion of MPTP to MPP+. Furthermore, it supports the conclusions of the DATATOP study.⁷⁴ Since we delayed treatment with deprenyl until 72 hours after the last MPTP dose, the finding that 61% of the cells that would have died without deprenyl treatment were rescued suggests that almost 100% of the cells which had not already died at the time of initiating treatment were saved. At present, we can only speculate on the mechanism of the deprenyl rescue. Although the rat values for percentage MAO-B inhibition for our two doses can be taken as rough guidelines, it is uncertain whether the 0.25 mg/kg dosage completely inhibited MAO-B action through a summation effect of the multiple doses. Hence, the rescue may or may not be mediated through MAO-B inhibition.

If the rescue is MAO-B inhibition-dependent then the data may point to glial participation. All of the MAO-B in the striatum is located in astroglia (see references above). If the rescue is in fact glial-dependent then it may not be limited to dopaminergic neurons since MAO-B is found in astroglia throughout the mammalian nervous system. The rescue of the MPTP-damaged DNSNs is similar to that which has been found after treatment with trophic factors or gangliosides.⁷⁵⁻⁷⁷ Glia are known to produce a number of trophic factors and one possible mechanism for a glial-mediated deprenyl action would be an increased production of a trophic factor in response to the treatment. Other mechanisms for rescue might be dependent upon a reduction in oxidative stress⁷⁸ or altered dopaminergic neurotransmission.^{70,71,79}

In conclusion, our studies of abiotrophy in a mouse model of PD illustrate the usefulness of the concept to understanding the time course of PD. Yet the studies show that the four components cannot necessarily be treated as independent variables and can be modulated by a variety of influences, some of which may offer new and important therapeutic vistas for PD and other conditions characterized by neuronal death.

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