[Frontiers in Bioscience 8, s826-837, September 1, 2003]

PRENATAL EXPOSURE TO THE BACTERIOTOXIN LIPOPOLYSACCHARIDE LEADS TO LONG-TERM LOSSES OF DOPAMINE NEURONS IN OFFSPRING: A POTENTIAL, NEW MODEL OF PARKINSON'S DISEASE.

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TABLE OF CONTENTS

1. ABSTRACT

The cause of Parkinson's disease (PD) is currently unknown. Although a genetic cause has been implicated in familial PD, the vast majority of cases are considered idiopathic. Environmental toxins have been implicated as a cause for PD by many investigators. Unfortunately, the magnitude of this exposure would likely need to be very high and as a result, would likely have been identified by the many epidemiological studies performed to date. Recently, we inadvertently realized that exposure to neurotoxins while still *in utero* may also represent a risk factor. Thus, exposure to the bacteriotoxin, lipopolysaccharide (LPS) during a critical developmental window in rats, leads to the birth of animals with fewer than normal dopamine (DA) neurons. This DA neuron loss is apparently permanent as it is still present in 16 months old animals (the longest period studied to date). Moreover, the loss of DA neurons seen in these animals increases with age thereby mimicking the progressive pattern of cell loss seen in human PD. The DA neuron loss is accompanied by reductions in striatal DA, increases in DA activity, and increased production of the pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-alpha). These are also characteristics of the PD brain. This model therefore shares many of the same characteristics with PD, and most importantly exhibits a slow, protracted loss of DA neurons – a characteristics of this animal model not found in other models. Interestingly, a common complication of pregnancy is a condition known as bacterial vaginosis (BV), which is known to produce increased levels of LPS and pro-inflammatory cytokines in the chorioamniotic environment of the fetus. This raises the interesting possibility that BV may be a risk factor for PD. The possibility that prenatal toxin exposure may contribute to the development of a neurodegenerative disease of the aged raises interesting new pathogenic questions and draws attention to the possibility that *in utero* exposure to neurotoxins may represent a here to fore unrecognized cause of PD.

2. THE ETIOLOGY OF PARKINSON'S DISEASE (PD)

Evidence links both genetic and environmental factors to the development of PD. Autosomal dominant inheritance of PD has been mapped to chromosomes 4 (alpha-synuclein) (1,2), 2p13 (PARK3), 4p15 (PARK4), 14q32.1 (SCA3), and 12q23-q24.1 (SCA2)(3). Autosomal recessive inheritance of PD has mapped to chromosomes 6q25.2-q27 (Parkin), and 1p35-p36 (PARK6, 7) (4-6). Although a genetic basis accounts for some cases of PD, the vast majority of PD is idiopathic and therefore of unknown etiology. This has led many to suggest that environmental factors are responsible. Exposure to agrochemicals such as pesticides/herbicides or copper, manganese, lead, and iron (7-9) may account for some cases, but a clear-cut relationship between these environmental factors and most cases of PD has not been established. Other environmental factors including exposure to viruses (post-encephalitic PD) (10) and consumption of foods thought to contain excitotoxins

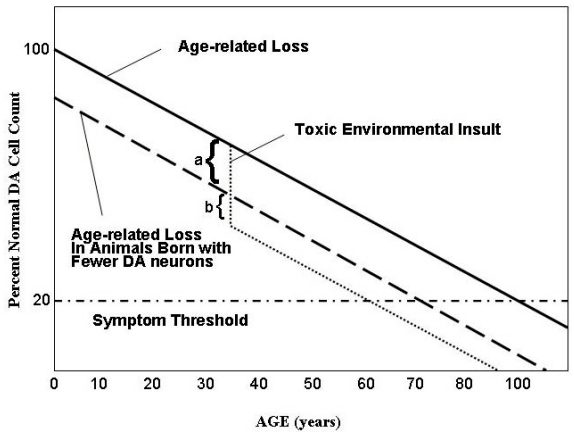

Figure 1. Depiction of the conventional hypothesis for Parkinson's disease. The solid line indicates the theoretical age-related loss of DA neurons that occurs in all individuals. The dotted line depicts the DA cell loss induced by a short-lived exposure to an environmental neurotoxin which kills a sub-population of those cells. The age related decline then brings that patient to the symptom expression threshold in later life. The dashed line indicates age-related DA neuron loss in patients born with fewer DA neurons. In normal individuals, the magnitude of the toxin exposure needed to satisfy the premise of the Calne-Langston hypothesis (Calne and Langston, 1983 (13)) is very large (a + b) and would likely be detected in epidemiological studies. In contrast, the magnitude of neurotoxin exposure needed in patients born with fewer than normal DA neurons would be significantly less (b).

(ALS-PD-Dementia Complex of Guam) have also been proposed (11). In the mid-1980s the involvement of environmental dopamine (DA) neurotoxins in PD was brought into focus when drug abusers inadvertently consumed a synthetic opioid compound eventually found to contain the DA specific neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)(12). As a result, Calne and Langston (13) hypothesized that an environmental neurotoxin, in combination with normal aging, was responsible for PD (figure 1). They proposed that toxin exposure killed DA neurons, but that the toxin-induced DA neuron loss was not, by itself, sufficient to bring the affected patient to the symptom threshold (figure 1 a; symptom threshold is widely believed to be loss of ~80% of the striatum's DA and ~50% of the DA neurons in the substantia nigra compacta (SNc)). They thus proposed that normal age-related losses of DA neurons resulted in further DA neuron loss sufficient to produce PD symptoms. In some respects this hypothesis has been borne out since abusers who took large dosages of MPTP developed symptoms within a week while others exposed to lower dosages, developed symptoms years later (14). Over the past 19 years, however, both major components of the Calne-Langston hypothesis have been challenged. Current thinking suggests that both environmental neurotoxins and aging are involved, but not as originally proposed.

3. ENVIRONMENTAL NEUROTOXINS AND PD

Numerous DA neurotoxins are known to exist and exposure to them has been hypothesized to increase the risk of PD. These chemicals can be synthetic or natural and many have been used as pesticides or herbicides (e.g., paraquat and maneb (14-18), rotenone (19), or dieldrin (20-22)). Exposure to these chemicals can be occupational, or alternatively, can arise as a result of consumption of well water contaminated with these substances. Indeed, several authors have argued that exposure to well water from agricultural communities where herbicides and insecticides are frequently used, increases the risk for developing PD (23,24). Other toxins are produced endogenously in the brain, often as a result of excess DA metabolism (e.g.: 6-hydroxydopamine (6OHDA)(25,26), cysteinyl adducts of DA(27-30), dopaldehyde (DOPAL) (31,32), N-methyl-norsalsolinol (33), N-methyl-salsolinol (34), tetrahydropapaveroline (35-37), and 1-benzyl-tetrahydroisoquinoline (38,39). Regardless of their source, all of these chemicals have been shown to reduce numbers of DA neurons in tissue culture, in animals, or both. Even though many of these chemicals have been found in the brains of PD patients, if they were responsible for PD, it is likely that epidemiological studies would have identified clusters of patients sharing that exposure as was the case with MPTP, post-encephalitic Parkinsonism, and the ALS-PD-Dementia Complex of Guam. It could be argued that the lessons learned from the ALS-PD-Dementia Complex of Guam as well as Post-encephalitic PD suggest that symptom expression can follow exposure by many decades making identification of environmental neurotoxins difficult. Even so, the failure to identify clusters of patients exposed to a single environmental toxin makes it unlikely that a single environmental neurotoxin can account for all cases of PD. This has led to hypotheses suggesting that multiple factors are responsible for PD and the Calne-Langston hypothesis would have to be revised accordingly. Regardless, whether or not exposure to environmental neurotoxins is a risk factor for PD, the Calne-Langston hypothesis also requires age-related losses of DA neurons to produce further cell loss and PD symptoms. Unfortunately, recent studies suggest that age-related loss of DA neurons does occur in humans, but that the pattern of cell loss is inconsistent with that typically seen in PD.

4. AGING AND DA NEURON LOSS

In humans, the number of DA neurons decreases with age (40,41). These studies are accompanied by an extensive literature demonstrating age-related indicators of DA neuron loss including loss of DA transporters (DAT) (42), striatal DA (43), DA receptors (44-46), and increased DA activity (43,47). Most of these age-related changes in DA markers generally exhibit a linear decline as seen in figure 1. The same changes (decreased nigral THir and DATir neurons) have also been reported in aged Rhesus monkeys (48). If normal aging were responsible for a progressive decline in DA neurons in a PD patient exposed to an environmental neurotoxin, then the loss of DA neurons in the preclinical and clinical phases of PD would similarly be linear. However, the rate of PD progression is

thought to be exponential early in the disease process (see extensive discussion by Agid, Y(49)) and a recent positron emission tomography (PET) study suggested that the degenerative process responsible for PD starts approximately 5-7 years prior to symptom expression and is more rapid than would be anticipated by normal aging (50). Moreover, the pattern of cell loss in the SN compacta (SNc) of PD patients appears to differ from the aged brain, by predominantly affecting the lateral SN and its ventral tier (41). The loss of DA in aged brain is similar in the caudate and the putamen nucleus with the caudal component of both nuclei being more affected than the rostral subdivisions. However, the levels of subregional DA metabolism, as measured by the homovanillic acid/dopamine ratio, in young individuals was found to be inversely correlated to the degree of subregional DA loss suffered by the individuals in the older age groups (43). These observations argue against normal aging as a contributor to PD. However, the focus on aging as it relates to PD pathogenesis has primarily been on loss of cell numbers. If we were to presume that PD is a consequence of exposure to environmental neurotoxins as well as age-related loss, then a neurotoxin could produce a more lateral loss of DA neurons and subsequent age-related losses would reduce the overall number of cells. This could account for the pattern of cell loss seen in the PD and still be consistent with the Calne-Langston hypothesis.

5. AGE AT EXPOSURE TO ENVIRONMENTAL TOXINS

Inherent within the Calne-Langston Hypothesis is the notion that exposure to environmental toxin(s) occurs during mid-life. This is presumably based on the assumption that an individual is most likely to be exposed to harmful DA neurotoxins as result of work-related activity, consumption of well water contaminated with neurotoxins, or exposure to infectious agents. However, what if exposure were to occur *in utero* and somehow interfere with the normal development of the DA neuron? An individual so exposed might be born with fewer than normal DA neurons and further, would be at increased risk for DA neuron loss resulting from future DA neurotoxin exposure. Together with age-related loss, this could lead to the pattern of cell loss seen in PD patients. More importantly, if individuals born with fewer than normal DA neurons did indeed exist and they were more susceptible to the effects of environmental toxins, then the level of exposure would likely be significantly lower than expected (b in figure 1). Low dose exposure would make it more difficult to detect epidemiologically, increasing the probability that environmental toxins are involved with the etiology of PD. In addition, exposure during development might include a set of entirely new neurotoxins that have not been considered previously. Recent data from our laboratory suggest that *in utero* toxin exposure may indeed serve as an entirely new and here to fore not considered risk factor for PD.

6. DA NEURON DEVELOPMENT

Our interest in exploring prenatal risk factors for PD developed serendipitiously. Soon after the seminal publication by Reynolds and Weiss (51) demonstrating that stem cells could successfully be isolated from the subventricular zone of the adult brain, we set out to identify cells with similar properties in the mesencephalon. Our intent was to find cells that were destined to become DA neurons, isolate them, expand and passage them, and then utilize them as an unlimited source of DA neurons for transplantation into patients with PD. For two years we were able to isolate and passage these cells, but regardless of the molecules used (e.g., neurotrophic factors, known growth factors, or tissue homogenates), we were unable to reliably induce their conversion into cells that expressed tyrosine hydroxylase (TH), a marker for DA neurons. This failure led to the search for a completely different set of signaling molecules. We reasoned that since hematopoietic cytokines normally regulate the expansion and lineage restriction of cells in bone marrow, they might perform similar functions within the CNS. We evaluated every cytokine then currently available and discovered that IL-1alpha and IL-1beta were able to induce the DA phenotype (52). We performed a clonal expansion of these mesencephalic progenitor cells and found that certain clones were more susceptible to the TH inducing effects of IL-1. We characterized this clone and showed that in response to IL-1, leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF), the cells exhibited several phenotypic markers of the DA neuron including TH, the DA transporter, DA, potassium-evoked DA release, and DOPA decarboxylase (DDC). Moreover, these cells reliably converted in high percentage to DA neurons following an *in vitro* five hour exposure to the cytokine "cocktail", survived implantation into 6-hydroxydopamine lesioned rats, and attenuated amphetamine-induced rotational asymmetry as effectively as freshly harvested ventral mesencephalic grafts (53). A similar conversion has now been performed in human mesencephalic progenitors (54). We subsequently attempted to modify IL-1 in the developing brain in an effort to demonstrate that this cytokine was centrally involved in the development of the nigro-striatal pathway. Our focus at that time was predominantly on IL-1 since our *in vitro* studies suggested that this molecule played a central role in regulating the expression of the DA phenotype during development. We therefore asked the question as "What would happen if we increased IL-1 levels in the fetal brain just prior to the development of DA neurons?" Our assumption was that we would produce a brain "hyper-innervated" with DA neurons. We observed the exact opposite.

7. *IN VIVO* LPS EXPOSURE REDUCES E14.5 THir CELLS IN PRIMARY MESENCEPHALIC CULTURES

Gravid female rats from embryonic (E) day 9 to E14 were injected i.p. with 1 mg/kg lipopolysaccharide (LPS). LPS (also called endotoxin) is a potent immunostimulant derived from the cell wall of Gram (-) bacteria. Optimal LPS signaling requires activation of the CD14 receptor and the Toll-like receptor (TLR4) which are expressed on monocytes and macrophages (55-57) as well as in brain cells (predominantly microglia) (56,57). The binding of LPS to its receptors is facilitated by LPS binding

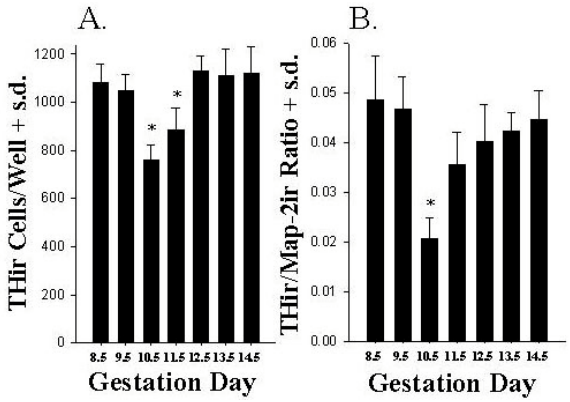

Figure 2. Prenatal LPS exposure at E10.5 reduces THir cell counts in mesencephalic cultures. Gravid rats received one i.p. LPS injection at various gestational days. The embryos were collected for mesencephalic cultures at E14.5 (animals received LPS at E14.5 were sacrificed 5 minutes after LPS injection and were used as a control group). Cultures were processed for tyrosine hydroxylase (TH) and microtubule associated protein 2 (Map-2) immunoreactive (ir) cells after three days. The greatest THir cell loss (a) and lowest THir/Map-2ir ratio (B) were observed in cultures prepared from animals exposed to LPS at E10.5 (*: $P<0.05$; bars represent mean and standard deviation).

protein (LBP) which binds LPS through recognition of lipid A and shuttles LPS onto the CD14 receptor which then couples with TLR 4 (57,58). Cells activated by LPS produce lysosomal enzymes, increase phagocytosis, secrete hydrolases, and release proinflammatory cytokines such as IL-1beta, TNF-alpha, IL-6, IFN-gamma, and other chemokines (59). Since LPS is widely used to increase proinflammatory cytokines and penetrates tissues readily, it seemed a logical choice for this experiment. At E14.5, the rats were sacrificed, the embryos removed, and the mesencephalic tissue harvested and cultured as previously described (60). Cells were grown for three days and fixed for TH and microtubule associated protein (Map-2; a general marker for neurons) immunocytochemistry. The number of TH immunoreactive (ir) cells and the ratio of THir cells to Map-2ir cells were used as dependent variables.

Significant THir cell loss ($F_{6,13} = 2.934$, $P=0.02$) as well as a decrease in the ratio of THir/Map-2ir cell counts ($F_{6,13}=4.69$, $P=0.01$) were observed (figure 2). The loss of THir cells (figure 2 A) and the reduction in the ratio (figure 2 B) were most pronounced in the cultures corresponding to the E10.5 LPS exposure (61). These data suggested that LPS was toxic to DA neurons *in vivo* and further, that the toxic effects observed were dependent upon gestational age. Since E11 is the earliest time that THir mRNA can be detected in the rat embryo (62), it appeared that LPS had its most pronounced effects when DA neurons were being born. Although IL-1beta was increased in these brains, its levels were significantly higher than that shown optimal for DA neuron induction in tissue culture. In fact, we had previously shown that the effects of IL-1beta on stem cell conversion exhibited an inverted U-shaped dose-response curve suggesting that higher levels of IL-1beta were toxic to normal DA neuron development (52). In addition, we had previously shown that other cytokines induced by exposure to LPS, were also toxic to DA neurons. Thus, we demonstrated that TNF-alpha could selectively kill DA neurons in tissue culture via apoptosis and that both the TNFR-1 and TNFR-2 receptors were present on young developing DA neurons (63). It was therefore possible that these pro-inflammatory cytokines participated in killing DA neurons instead of stimulating the anticipated increase in cell number.

8. PRENATAL LPS EXPOSURE REDUCES DA NEURONS

Although the results from the previous study were suggestive of LPS *in vivo* toxicity, they represented an artificial environment (*in vivo* toxin with effects assessed *in vitro*). In addition, it was possible that the effects LPS had on the growth of DA neurons in tissue culture were transient or represented a developmental delay, but that the actual number of DA neurons present by the time of birth were normal. We thus examined the effects of LPS *in vivo* by studying prenatally exposed offspring at various postnatal (P) days. All studies completed to date have shown the same attenuation in DA neuron counts.

All studies have involved making a single i.p. LPS injection of 10,000 endotoxin units (EU)/kg at E10.5. The dams tolerate the procedure very well since the dose used is actually quite low relative to those used to study sepsis and fever. As can be seen from table 1, the THir cell loss is approximately 30% in very young animals and appears to increase progressively with age. Several different studies have been run to date with the longest survival time being 16 months. In table I four groups of animals have been depicted. The data from animals sacrificed at P10 and P21 were from two separate groups and all the cell counts were assessed in a stereological fashion. The cell counts from the P120 and P480 groups were from litter mates. Figure 3, shows typical TH staining of the mesencephalons from control animals (figure 3 A) and the animals exposed to LPS prenatally (figure 3 B). Note that the cell loss was most pronounced in the lateral regions of the SNc as well as its ventral tier. Demonstrating reduced cell counts in these older animals following prenatal exposure is important since this time is well past the primary and secondary phases of natural apoptotic cell death (DA neuron culling peaks at P2 and again at P14 (64)) suggesting that LPS does not produce a developmental delay, but rather a lasting cell death. The cell loss seen in these animals was also associated with reduced striatal DA as well as elevated DA activity ([HVA)]/[DA]) suggesting that those DA neurons remaining were synthesizing and metabolizing DA more rapidly. Such increases in DA activity are almost always seen in patients and animals with DA neuron loss and are associated with increased production of free radicals. Increases in reactive oxygen species (ROS) are a common finding in PD brain and may contribute to further DA neuron loss and the progression of disease (65,66).

Table 1. Comparison of SN THir cell counts, striatal DA, and DA activity in prenatally LPS exposed rats and HBSS exposed controls

	TNF-alpha (% change from control)		IL 1-beta (% change from control)	
Age at Sacrifice	**Mesencephalon**	**Striatum**	**Mesencephalon**	**Striatum**
P10[98]	189% (9.00 ± 5.15)	940% (22.78 ± 7.44)	170% (3.55 ± 1.56)	173% (8.44 ± 3.20)
P21[59]	92% (15.18 ± 5.95)	100% (18.16 ± 5.46)	–	--
P120	64% (71.49 ± 6.16)	--	n.d.	n.d.
P480	319% (57.95 ± 22.63)	352%(21.25 ± 16.73)	n.d.	n.d

The estimation of the total number of THir neurons in the SN was performed using the computerized optical dissector method. This method allowed for the stereological estimation of THir cells in the entire structure independent of size, shape, orientation, tissue shrinkage or anatomical level using MicroBrightField software (61). If a percent change is indicated it is $p < 0.05$; n=6-8/treatment group; the numbers following percent change are the mean and S.D. of the control group.

Table 2. Increase in pro-inflammatory cytokines in animals exposed to LPS prenatally relative to HBSS animals

	TNF-alpha (% change from control)	IL 1-beta (% change from control)		
Age at Sacrifice	**Mesencephalon**	**Striatum**	**Mesencephalon**	**Striatum**
P10(102)	189% (9.00 ± 5.15)	940% (22.78 ± 7.44)	170% (3.55 ± 1.56)	173% (8.44 ± 3.20)
P21(61)	92% (15.18 ± 5.95)	100% (18.16 ± 5.46)	–	--
P120	64% (71.49 ± 6.16)	--	n.d.	n.d.
P480	319% (57.95 ± 22.63)	352%(21.25 ± 16.73)	n.d.	n.d

If a percent change is indicated it is $p < 0.05$; -- = $p > 0.05$; n.d. = not detectable; n = 6-8 animals/treatment; the numbers following percent change are the mean and S.D. of the control group.

In addition to losses in DA neurons, prenatal exposure to a single dose of LPS at E10.5 leads to elevations in pro-inflammatory cytokines, especially TNF-alpha (table 2). Although IL-1 beta was increased in early post-natal life, this pro-inflammatory cytokine returned to normal levels by P21. In contrast, TNF-alpha levels were not only elevated in early post-natal life, they remained elevated throughout life. This finding is significant since TNF-alpha has been shown to be elevated in the brains of patients dying with PD (67,68). It is also noteworthy that TNF-alpha is toxic to DA neurons. The increases in ROS produced by augmented DA activity together with higher levels of TNF-alpha could combine to produce a self-perpetuating cycle of DA cell loss thereby contributing to progressive degeneration – the hall mark of PD. Indeed, inflammatory processes, such as gliosis and increased TNF-alpha, are associated with several chronic neurodegenerative disorders such as PD, Alzheimer's disease, and amyotrophic lateral sclerosis (67-69).

9. PRENATAL INFECTION, PROINFLAMMATORY CYTOKINES AND BACTERIAL VAGINOSIS (BV)

Although these data were encouraging and suggested that prenatal exposure to LPS and pro-inflammatory cytokines might lead to animals born with fewer than normal DA neurons, the clinical relevance of such a model was not apparent. As we pursued this concept, we discovered an extensive literature on a common complication of pregnancy called Bacterial Vaginosis (BV). BV commonly occurs in pregnancy (incidence of 14%) (70) and is widely believed to lead to serious pre- and perinatal complications including pre-term parturition, low birth weight and fetal demise (71,72). BV is generally associated with the overgrowth of *Gardnerella vaginalis*, a Gram variable bacteria as well as several Gram (-) bacteria including Eschericia coli (E. coli) which flourish in the vaginal environment when its pH exceeds 4.5 as often occurs in pregnancy (73). BV often does not produce signs of overt infection aside from a vaginal discharge and, as a result, generally goes untreated. Whether it is symptomatic or asymptomatic, an extensive literature reveals that BV is clearly associated with increased levels of IL-1alpha and beta, IL-6, and TNF-alpha in the chorioamniotic environment and is presumed to be the leading cause of premature delivery and low birth weight (74-76). For instance, virtually all women with bacterial intra-amniotic infection have high levels of the cytokines TNF-alpha, IL-1beta, and IL-6 in their amniotic fluid (74-76). These cytokines are proposed to trigger pre-term delivery by stimulating the synthesis of prostaglandins by intrauterine tissues which soften the uterine cervix, thus leading to the initiation of labor. LPS has been detected in the amniotic fluid of mothers with bacterial complications as well (77), and is proposed to be the predominant stimulus in promoting the production of cytokines and prostaglandins at the feto-maternal interface (74,78-82). It is also assumed that maternal LPS induces cytokine production by fetal tissues (72,83-86). In addition to premature delivery and low birth weight, BV has been linked to numerous neurological disorders including white matter damage (periventricular leukomalacia), intraventricular hemorrhage, and cerebral palsy (84-86).

The existence of BV and its common incidence in pregnancy suggests that exposure to LPS in utero is a realistic possibility. The fact that it is strongly associated with CNS disorders further suggests that BV could readily produce the effects on DA neuron development hypothesized to occur in the LPS animal model. However, it is important to recognize that

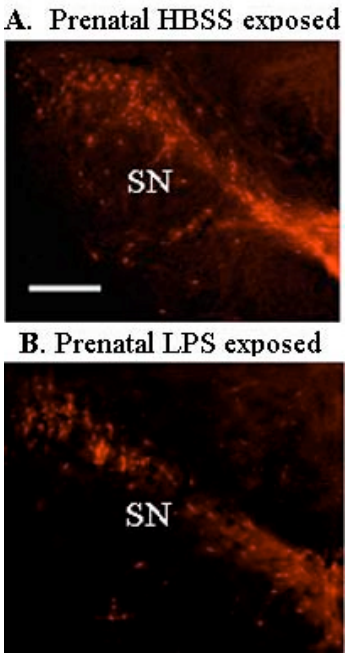

Figure 3. Typical TH immunostaining of the mesencephalons from prenatally HBSS exposed animal (A) and prenatally LPS exposed (B) (at E10.5). Note that the cell loss was most pronounced in the lateral regions of the SNc as well as its ventral tier (bar equals to 0.5 mm).

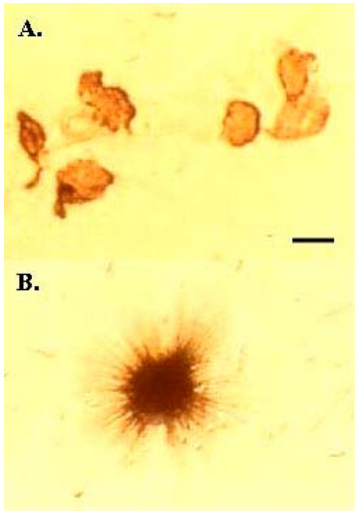

Figure 4. Microglia were isolated from P1 rat pups as previously described (101). The cultures were grown out for 2 weeks and then exposed to LPS (1,000 EU/ml) or HBSS. After 72 hours the cultures were processed for GFAP and Ox-42 immunostaining. The cultures were virtually microglia pure since only a few GFAP positive astrocytes were detected (data not shown). Cultures exposed to HBSS contained mostly Ox-42-ir cells that had smooth borders indicative of normal resting microglia (A). In contrast, the cultures exposed to LPS had a high percentage of microglia that had uneven borders with fine spike-like processes indicative of activation (B) (bar equals to 5 μm).

the effects of LPS on DA neuron development in the rat only occurred following exposure at E10.5 and to a lesser extent, E11.5. This would suggest that BV would only be a potential risk factor for PD when it was present when DA neurons are being born (week 7-9 in humans). Thus, even though the incidence of BV is high during pregnancy, the potential risk of developing PD later in life would be considerably lower. At this time, the incidence of BV during the early stages of pregnancy is unknown.

10. CYTOKINES AND PD

Although the potential involvement of BV is new, a considerable literature exists on the involvement of pro-inflammatory cytokines and DA toxicity. Several studies have now shown that the pro-inflammatory cytokines TNF-alpha as well as IL-1beta are elevated in the SNc of patients with PD (68,87-90). As predicted by their function, the increases in these pro-inflammatory cytokines were coupled with increases in apoptosis-related proteins and oxidative stress in PD patients (91,92). In animals, intracerebral ventricle (i.c.v.) injection of LPS has been shown to reduce TH and DA content in the SNc (93) and in mesencephalic cultures (94,95). Similarly, TNF-alpha and IL-1beta are neurotoxic to THir cells in the E14 rat fetus (63,96). A recent finding also reported that administration of IL-1beta at birth reduced brain DA content in adulthood (97). However, if pro-inflammatory cytokines were involved in the pathogenesis of PD, they would have to kill DA neurons specifically and do so more readily in an aged brain. It is difficult to envision such selectivity given the generalized inflammatory response associated with BV. Interestingly, the highest density of microglia in the brain is located within the mesencephalon in rats (98) (whether this is true or not in humans has not yet been examined). Since microglia are considered a major source of production of pro-inflammatory cytokines (99), it becomes possible that regional heterogeneity in the distribution of this cell could lead to preferential regional neurodegeneration.

In order to begin to assess the potential involvement of microglia in DA neurotoxicity, we evaluated the effects of LPS on microglia cultures harvested from rat brain. Microglia were isolated from P1 rat brain as previously described and cultured for two weeks. They were then exposed to various concentrations of LPS (0.1 to 1,000 EU/ml) for 24 hours. The cultures were subsequently fixed and immunostained for the microglia marker Ox42. The supernatants were assessed for TNF-alpha using ELISA as described in the R and D kit instructions (61). LPS increased TNF-alpha production in a dose-dependent fashion ($F_{1,9}$=914.254; $P<0.001$; TNF-alpha ranges from 0 to 196 pg/ml). Moreover, examination of the cultures immunostained for Ox42 revealed a dramatic increase in the number of activated microglia (activated microglia shown in figure 4 B). These data suggest that LPS induces increases in TNF-alpha in CNS microglia associated with activation of microglia, and that microglia could be the source of increases in pro-inflammatory cytokines within the CNS following exposure to LPS. Whether or not activated microglia remains the

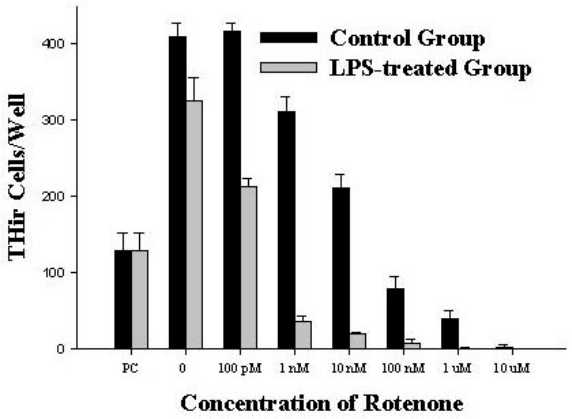

Figure 5. LPS sensitized primary mesencephalic cultures to subsequent exposure to rotenone. Primary mesencephalic cultures were harvested from E14.5 rat fetuses. After 72 hours in complete media (CM) containing 10%fetal calf serum (FCS), they were exposed to a fixed concentration of LPS (1,000 EU/ml) for an additional 72 hours. The media was then changed and the cultures were subsequently exposed to CM alone or CM containing various concentrations of rotenone (100 pM-10 micro M). After an additional 72 hours, the cultures were processed for tyrosine hydroxylase immunochemistry and the numbers of THir cells were counted.

source of increased TNF-alpha production in the months following LPS exposure remains to be established. However, if as the *in vivo* data shows, TNF-alpha remains elevated throughout life, and these animals have reduced numbers of DA neurons to begin with, then exposure to a DA neurotoxin later in life should produce a more profound DA neuron loss. Preliminary studies in our laboratory are consistent with this hypothesis.

11. DA NEUROTOXIN TREATMENT FOLLOWING LPS EXPOSURE

The effect of prior exposure to LPS on the subsequent effects of DA neurotoxins has been explored both *in vitro* and *in vivo*. Primary mesencephalic cultures harvested from E14 rat fetuses were cultured as previously described. After 72 hours in complete media (CM) containing 10%fetal calf serum (FCS), they were exposed to a fixed concentration of LPS (1,000 EU/ml) for an additional 72 hours. A dose of 1,000 EU/ml LPS was chosen here because previous studies had shown that it had a minimal effect on the growth of THir cells in culture. The media was then changed and the cultures were subsequently exposed to CM alone or CM containing various concentrations of rotenone (100 pM-10 micro M). After an additional 72 hours, the cultures were processed and assessed for the number of THir expressing neurons as previously described.

Rotenone, produced a dose-dependent decrease in THir cells in culture ($F_{1,13}$=59.951; P<0.001) (figure 5). The 100 nM concentration produced a statistically significant loss of cells, and at concentrations greater than 1 micro M, virtually all the THir cells were lost. Since rotenone is a complex I inhibitor with a mechanism similar to that of the known DA neurotoxin MPTP (19), its toxicity was not unexpected. In contrast, prior exposure to LPS produced a dramatic left-ward shift in the toxicity curve. Thus, in cultures pre-exposed to LPS and subsequently exposed to rotenone, concentrations as low as 100 pM significantly reduced the THir cell counts. As expected, pretreatment with the low concentration of LPS used, had a minimal, non-significant effect on the number of THir cells in the cultures (18% decrease). However, the combined effects of pre-treatment with LPS and rotenone appeared to have a synergistic effect on THir cell counts as reflected by the significant interaction term ($F_{1,6}$=4.735; P=0.008). Although these results should only be considered preliminary and the effects of the two toxins on other cell types in the cultures must be assessed, they do suggest that prior exposure to LPS sensitizes DA neurons to the effects of subsequent toxin exposure. Again however, a number of factors unique to the tissue culture environment could have contributed to this effect. We therefore evaluated the effects of rotenone in 8 month old animals that had been exposed to LPS prenatally in a small pilot study to determine if prenatal exposure to LPS sensitized adult animals to the effects of DA toxins.

Gravid female rats (n=21) were treated with 10,000 EU/kg LPS (n=11) or Hank's Balanced Salt Solution (HBSS; n=10) *in utero* at E10.5. The females gave birth at ~E21.5 and the apparently normal pups were allowed to mature. One animal from each litter was then passed to post-natal treatment at 7.5 months. Animals were continuously infused into the right jugular vein via Alzet mini-pump with either HBSS or rotenone (1.5 mg/kg/day) for 14 days as previously described (19). After 14 days, the animals were sacrificed, the brains removed and fixed, and the mesencephalons immunostained for TH as described previously.

Unfortunately, the data from this study is only suggestive, since an unanticipated high mortality was seen in the rotenone infused animals exposed to LPS prenatally. Only three of the animals in this group survived the protocol and two others were sacrificed prematurely due to significant morbidity. Only two animals exposed to HBSS prenatally and rotenone at 7.5 months died (within 72 hours of the surgery) and the other 8 animals tolerated the procedure without problem. Examination of the mesencephalic sections from animals exposed to LPS prenatally and infused with rotenone after 7.5 months revealed a more profound THir cell loss relative to animals exposed to LPS and saline. Moreover, the TH immunostaining in the rotenone animals exposed to LPS prenatally exhibited a diffuse pattern consistent with severe necrosis, massive cell loss and inflammation (figure 6, C and **D**). Although this study must be repeated, the preliminary findings appear to support those from tissue culture and suggest that prenatal exposure to LPS renders the animals so exposed more sensitive to the effects of DA neurotoxins in later life. A recent study by Gao et al. has demonstrated synergistic toxic effects of LPS and rotenone

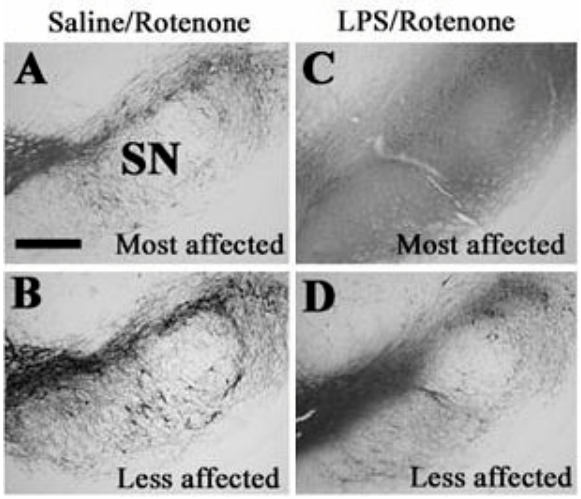

Figure 6. Photo-micrographic depiction of the substantia nigra of 7 month old rats exposed to prenatal HBSS (A and B) and LPS (C and D) followed by postnatal jugular rotenone infusion. The animals exposed to LPS prenatally and rotenone postnatally (LPS/Rotenone) had profound cell loss and diffuse THir staining indicative of severe necrosis (C and D) (bar equals to 0.5 mm).

in rat mesencephalic cultures and this synergy was likely mediated by NADPH oxidase (100). This is in agreement with our findings.

12. CONCLUSION AND PERSPECTIVES

12.1. Prenatal LPS as an Animal Model of PD

The data completed to date demonstrates that prenatal exposure to a low dose of LPS leads to the birth of animals born with fewer than normal DA neurons. This DA neuron loss appears to increase with age. This progressive DA neuron loss is associated with reduced levels of striatal DA and increased DA activity. In addition, the pattern of DA neuron loss is similar to that seen in patients with PD, since the magnitude of loss is most pronounced in the lateral SNc and its ventral tier. As is true in PD, the loss of DA neurons is accompanied by increases in TNF-alpha as well as oxidized protein. Taken together, this animal model shares many of the same characteristics with PD (microglial activation and gliosis are currently under investigation). More importantly, the progressive cell loss seen in these animals is slow and protracted. Although many animal models have been shown to produce the characteristics of PD seen in the prenatal LPS model, none have been able to produce a progressive continued loss of DA neurons in the absence of continued toxin exposure. This unique feature of the LPS model lends itself to a variety of types of studies that are not practical in the other models where the toxin-induced DA neuron loss occurs over a few days or at best a few weeks. In addition, the slow progressive nature of the DA neuron loss in the LPS will enable us to better identify the compensatory changes of the nigro-striatal pathway to the gradual loss of DA neurons. The characterization of these changes will undoubtedly lend insight into the processes that accompany DA neuron loss in PD.

12.2. Prenatal LPS as a Model of BV

The potential link between prenatal LPS exposure and PD depends upon acceptance of the assumption that BV can indeed produce the types of changes seen with i.p. LPS treatment. We recently completed a simple study to address this important issue. If prenatal LPS exposure is to be considered a relevant risk factor for PD and our proposed animal model representative of that disease, we should be able to demonstrate that BV is capable of producing DA neuron loss in rats. We mixed matrigel plugs with a solution containing LPS to achieve 10,000 EU/kg. We then inserted the plugs into the vaginas of four gravid females at E11. Control rats had matrigel plugs saturated with HBSS inserted. We then monitored TNF-alpha in the fetal brains at E15. Mesencephalic TNF-alpha levels were increased from 20.70 ± 6.08 pg/mg protein in control animals to 30.02 ±7.88 pg/mg protein in the animals exposed to vaginal LPS ($P<0.03$, n=3 gravid female rats/group; five embryos from each female were studied). Although this increase is not nearly as high as the one seen following i.p. LPS treatment, it does clearly demonstrate that the LPS produced as a consequence of BV can indeed penetrate the chorioamniotic environment and elevate TNF-alpha in brain. Cultures of the mesencephalons from these embryos also demonstrated a toxic effect from LPS matrigel exposure during prenatal development. Thus, in mesencephalic cultures derived from fetuses of females exposed vaginally to HBSS, the average THir cell counts in these cultures was 1373 ± 270.9 cells whereas the cultures from fetuses exposed to matrigel plugs containing LPS had only 1071± 106.6 cells (*t* student test, $p<0.05$). We have considered the possibility of actually infecting the pregnant mother's vagina with Gram (-) bacteria, but feel we would not have control over dose. Rather, our intent with these studies was to demonstrate that vaginal LPS can reproduce the effects we see with i.p. LPS and then continue to use the i.p. model so that we can have better control over exposure dosage.

12.3. Implications of the LPS Model for the Pathogenesis of PD

The data on the prenatal LPS model collected thus far when considered within the context of BV, suggests that BV might be a risk factor for PD. The possibility that a prenatal toxin could be a risk factor for PD has not been explored previously. If indeed a group of individuals are born with fewer than normal DA neurons as a consequence of BV or exposure to some other type of toxin *in utero*, then the environmental hypothesis of PD would have to be revised to include epigenetic factors. Moreover, if, as our very preliminary data suggests, animals exposed to LPS prenatally are more susceptible to exposure to environmental neurotoxins later in life, then the level of exposure to those neurotoxins would be significantly reduced. Thus, exposure to other toxins known to kill DA neurons, (e.g., dieldrin) could be significantly lower than previously thought increasing the possibility that exposure to this widely distributed toxin may contribute to PD. Indeed, if it is assumed that patients are born with fewer than normal DA neurons, and that normal age-related declines occur as originally assumed by the Calne-Langston hypothesis, it is readily apparent that

the degree of neurotoxin exposure could be significantly lower (figure 1b). This would imply that all environmental DA neurotoxins thus far discovered are more involved in the pathogenesis of PD than previously thought. Unfortunately, developing a rational strategy to assess the potential involvement of BV in PD is difficult given the logistical problems associated with following patients born to mothers who had BV from birth to old age. However, it is hoped that the LPS animal model will begin to generate an appreciation that exposure to bacterotoxins and other types of toxins *in utero* should be considered when attempting to determine the potential causes for PD.

13. ACKNOWLEDGMENTS

The authors wish to thank all of those organizations that contributed funding to the exploration and initial development of this concept including The Blowitz-Ridgeway Foundation, The American Parkinson's Disease Foundation (APDA), the Department of Defense (DAMD17-98-1-8629 and DAMD17-01-1-0771), NIEHS (ES10776), and NINDS (NS0 45316).

14. REFERENCES

1. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045-7 (1997)
2. Nussbaum RL, Polymeropoulos MH. Genetics of Parkinson's disease. *Hum.Mol.Genet*. 6:1687-91 (1997)
3. Gasser T. Overview of the genetics of parkinsonism. *Adv.Neurol*. 91:143-52 (2003)
4. Gasser T. Genetics of Parkinson's disease. *Ann.Neurol.* 44:S53-S57 (1998)
5. Mouradian MM. Recent advances in the genetics and pathogenesis of Parkinson disease. *Neurology* 58:179-85 (2002)
6. Steece-Collier K, Maries E, Kordower JH. Etiology of Parkinson's disease: Genetics and environment revisited. *Proc.Natl.Acad.Sci.U.S.A* 99:13972-4 (2002)
7. Hubble JP, Cao T, Hassanein RE, Neuberger JS, Koller WC. Risk factors for Parkinson's disease. *Neurology* 43:1693-7 (1993)
8. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG et al. Occupational exposures to metals as risk factors for Parkinson's disease. *Neurology* 48:650-8 (1997)
9. Rybicki BA, Johnson CC, Uman J, Gorell JM. Parkinson's disease mortality and the industrial use of heavy metals in Michigan. *Mov Disord*. 8:87-92 (1993)
10. Williams A, Houff S, Lees A, Calne DB. Oligoclonal banding in the cerebrospinal fluid of patients with postencephalitic Parkinsonism. *J.Neurol.Neurosurg.Psychiatry* 42:790-2 (1979)
11. Cox PA, Sacks OW. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. *Neurology* 58:956-9 (2002)
12. Snyder SH, D'Amato RJ. MPTP: a neurotoxin relevant to the pathophysiology of Parkinson's disease. The 1985 George C. Cotzias lecture. *Neurology* 36:250-8 (1986)
13. Calne DB, Langston JW. Aetiology of Parkinson's disease. *Lancet* 2:1457-9 (1983)
14. Vingerhoets FJ, Snow BJ, Tetrud JW, Langston JW, Schulzer M, Calne DB. Positron emission tomographic evidence for progression of human MPTP- induced dopaminergic lesions. *Ann.Neurol*. 36:765-70 (1994)
15. Spencer PS, Ludolph AC, Kisby GE. Are human neurodegenerative disorders linked to environmental chemicals with excitotoxic properties? *Ann.N.Y.Acad.Sci*. 648:154-60 (1992)
16. Langston JW, Ballard P. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. *Can.J.Neurol.Sci*. 11:160-5 (1984)
17. Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res*. 823:1-10 (1999)
18. Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. *J.Neurosci*. 20:9207-14 (2002)
19. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat.Neurosci*. 3:1301-6 (2002)
20. Corrigan FM, Wienburg CL, Shore RF, Daniel SE, Mann D. Organochlorine insecticides in substantia nigra in Parkinson's disease. *J.Toxicol.Environ.Health* 59:229-34 (2002)
21. Kitazawa M, Anantharam V, Kanthasamy AG. Dieldrin-induced oxidative stress and neurochemical changes contribute to apoptopic cell death in dopaminergic cells. *Free Radic.Biol.Med*. 31:1473-85 (2001)
22. Fleming L, Mann JB, Bean J, Briggle T, Sanchez-Ramos JR. Parkinson's disease and brain levels of organochlorine pesticides. Ann.*Neurol*. 36:100-3 (1994)
23. Le Couteur DG, Muller M, Yang MC, Mellick GD, McLean AJ. Age-environment and gene-environment interactions in the pathogenesis of Parkinson's disease. *Rev.Environ.Health* 17:51-64 (2002)
24. Ritz B, Yu F. Parkinson's disease mortality and pesticide exposure in California 1984- 1994. *Int.J.Epidemiol*. 29:323-9 (2000)
25. Pezzella A, d'Ischia M, Napolitano A, Misuraca G, Prota G. Iron-mediated generation of the neurotoxin 6-hydroxydopamine quinone by reaction of fatty acid hydroperoxides with dopamine: a possible contributory mechanism for neuronal degeneration in Parkinson's disease. *J.Med.Chem.* 40:2211-6 (1997)
26. Linert W, Herlinger E, Jameson RF, Kienzl E, Jellinger K, Youdim MB. Dopamine, 6-hydroxydopamine, iron, and dioxygen--their mutual interactions and possible implication in the development of Parkinson's disease. *Biochim.Biophys.Acta* 1316:160-8 (1996)
27. Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B. Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. *J.Neurochem*. 71:2112-22 (1998)

28. Montine TJ. Endogenous brain catechol thioethers in dopaminergic neurodegeneration. Neurotoxic Factors in PD and Related Disorders, *Kluwer-Plenum Publ, NYC* 155-66 (2000)
29. Shen XM, Li H, Dryhurst G. Oxidative metabolites of 5-S-cysteinyldopamine inhibit the alpha- ketoglutarate dehydrogenase complex: possible relevance to the pathogenesis of Parkinson's disease. *J.Neural Transm*. 107:959-78 (2000)
30. Li H, Shen XM, Dryhurst G. Brain mitochondria catalyze the oxidation of 7-(2-aminoethyl)-3,4- dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxyli c acid (DHBT-1) to intermediates that irreversibly inhibit complex I and scavenge glutathione: potential relevance to the pathogenesis of Parkinson's disease. *J.Neurochem*. 71:2049-62 (1998)
31. Lamensdorf I, Eisenhofer G, Harvey-White J, Hayakawa Y, Kirk K, Kopin IJ. Metabolic stress in PC12 cells induces the formation of the endogenous dopaminergic neurotoxin, 3,4-dihydroxyphenylacetaldehyde. *J.Neurosci.Res*. 60:552-8 (2000)
32. Burke WJ, Li SW, Schmitt CA, Xia P, Chung HD, Gillespie KN. Accumulation of 3,4-dihydroxyphenylglycolaldehyde, the neurotoxic monoamine oxidase A metabolite of norepinephrine, in locus ceruleus cell bodies in Alzheimer's disease: mechanism of neuron death. *Brain Res*. 816:633-7 (1999)
33. Moser A, Kompf D. Presence of methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines, derivatives of the neurotoxin isoquinoline, in parkinsonian lumbar CSF. *Life Sci*. 50:1885-91 (1992)
34. Maruyama W, Sango K, Iwasa K, Minami C, Dostert P, Kawai M et al. Dopaminergic neurotoxins, 6,7-dihydroxy-1-(3', 4'-dihydroxybenzyl)- isoquinolines, cause different types of cell death in SH-SY5Y cells: apoptosis was induced by oxidized papaverolines and necrosis by reduced tetrahydropapaverolines. *Neurosci.Lett*. 291:89-92 (2000)
35. Maruyama W, Strolin-Benedetti M, Naoi M. N-methyl(R)salsolinol and a neutral N-methyltransferase as pathogenic factors in Parkinson's disease. *Neurobiology (Bp)* 8:55-68 (2000)
36. Sango K, Maruyama W, Matsubara K, Dostert P, Minami C, Kawai M et al. Enantio-selective occurrence of (S)-tetrahydropapaveroline in human brain. *Neurosci.Lett*. 283:224-6 (2000)
37. Surh Y. Tetrahydropapaveroline, a dopamine-derived isoquinoline alkaloid, undergoes oxidation: implications for DNA damage and neuronal cell death. *Eur.J.Clin.Invest* 29:650-1 (1999)
38. Kotake Y, Tasaki Y, Makino Y, Ohta S, Hirobe M. 1-Benzyl-1,2,3,4-tetrahydroisoquinoline as a parkinsonism-inducing agent: a novel endogenous amine in mouse brain and parkinsonian CSF. *J.Neurochem*. 65:2633-8 (1995)
39. Kotake Y, Yoshida M, Ogawa M, Tasaki Y, Hirobe M, Ohta S. Chronic administration of 1-benzyl-1,2,3,4-tetrahydroisoquinoline, an endogenous amine in the brain, induces parkinsonism in a primate. *Neurosci.Lett*. 217:69-71 (1996)
40. McGeer PL, Itagaki S, Akiyama H, McGeer EG. Rate of cell death in parkinsonism indicates active neuropathological process. *Ann.Neurol*. 24:574-6 (1988)
41. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 114 (Pt 5):2283-301 (1991)
42. Ma SY, Ciliax BJ, Stebbins G, Jaffar S, Joyce JN, Cochran EJ et al. Dopamine transporter-immunoreactive neurons decrease with age in the human substantia nigra. *J.Comp Neurol*. 409:25-37 (1999)
43. Kish SJ, Shannak K, Rajput A, Deck JH, Hornykiewicz O. Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. *J.Neurochem*. 58:642-8 (1992)
44. Wang Y, Chan GL, Holden JE, Dobko T, Mak E, Schulzer M et al. Age-dependent decline of dopamine D1 receptors in human brain: a PET study. *Synapse* 30:56-61 (1998)
45. Rinne JO, Hietala J, Ruotsalainen U, Sako E, Laihinen A, Nagren K et al. Decrease in human striatal dopamine D2 receptor density with age: a PET study with [11C] raclopride. *J.Cereb.Blood Flow Metab* 13:310-4 (19963)
46. Antonini A, Leenders KL, Reist H, Thomann R, Beer HF, Locher J. Effect of age on D2 dopamine receptors in normal human brain measured by positron emission tomography and 11C-raclopride. *Arch.Neurol*. 50:474-80 (1993)
47. Calne DB, Zigmond MJ. Compensatory mechanisms in degenerative neurologic diseases. Insights from parkinsonism. *Arch.Neurol*. 48:361-3 (1991)
48. Emborg ME, Ma SY, Mufson EJ, Levey AI, Taylor MD, Brown WD et al. Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. *J Comp Neurol*. 401:253-65 (1998)
49. Agid Y. Parkinson's disease: pathophysiology. *Lancet* 337:1321-4 (1991)
50. Morrish PK, Rakshi JS, Bailey DL, Sawle GV, Brooks DJ. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. *J.Neurol.Neurosurg.Psychiatry* 64:314-9 (1998)
51. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255:1707-10 (1992)
52. Ling ZD, Potter ED, Lipton JW, Carvey PM. Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. *Exp.Neurol*. 149:411-23 (1998)
53. Carvey PM, Ling ZD, Sortwell CE, Pitzer MR, McGuire SO, Storch A et al. A clonal line of mesencephalic progenitor cells converted to dopamine neurons by hematopoietic cytokines: a source of cells for transplantation in parkinson's disease. *Exp.Neurol*. 171:98-108 (2001)
54. Storch A, Paul G, Csete M, Boehm BO, Carvey PM, Kupsch A et al. Long-term proliferation and dopaminergic differentiation of human mesencephalic neural precursor cells. *Exp.Neurol*. 170:317-25 (2001)
55. Sweet MJ, Hume DA. Endotoxin signal transduction in macrophages. *J.Leukoc.Biol*. 60:8-26 (1996)
56. Kielian TL, Blecha F. CD14 and other recognition molecules for lipopolysaccharide: a review. *Immunopharmacology* 29:187-205 (1995)
57. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J.Biol.Chem*. 274:10689-92 (1999)

58. Fenton MJ, Golenbock DT. LPS-binding proteins and receptors. *J.Leukoc.Biol*. 64:25-32 (1998)
59. Paul WE. Fundamental Immunology. *Lippincott-Raven, Philadelphia, PA* (1998)
60. Ling ZD, Pieri SC, Carvey PM. Comparison of the Neurotoxicity of Dihydroxyphenylalanine (dopa) Stereoisomers in Cultured Dopamine Neurons. *Clin.Neuropharmacol*. 19:360-5 (1996)
61. Ling ZD, Gayle DA, Ma SY, Lipton JW, Tong CW, Hong JS et al. In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Mov Disord*. 17:116-24 (2002)
62. Specht LA, Pickel VM, Joh TH, Reis DJ. Light-microscopic immunocytochemical localization of tyrosine hydroxylase in prenatal rat brain. I. Early ontogeny. *J.Comp Neurol*. 199:233-53 (1981)
63. McGuire SO, Carvey PM, Ling ZD. Tumor necrosis factor is toxic to mesencephalic dopamine neurons. *Exp.Neurol*. 169:219-30 (2001)
64. Oo TF, Burke RE. The time course of developmental cell death in phenotypically defined dopaminergic neurons of the substantia nigra. *Brain Res.Dev.Brain Res*. 98:191-6 (1997)
65. Giasson BI, Ischiropoulos H, Lee VM, Trojanowski JQ. The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. *Free Radic.Biol.Med*. 32:1264-75 (2002)
66. Bennett JP. Free radicals, oxidative stress and the origin of Parkinson's disease. *J.Neurol.Sci*. 170:75-6 (1999)
67. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci.Lett*. 172:151-4 (1994)
68. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci.Lett*. 165:208-10 (1994)
69. McGeer PL, McGeer EG. Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. *Alzheimer Dis.Assoc.Disord*. 12 Suppl 2:S1-S6 (1998)
70. Thorsen P, Jensen IP, Jeune B, Ebbesen N, Arpi M, Bremmelgaard A et al. Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. *Am.J.Obstet.Gynecol*. 178:580-7 (1998)
71. Purwar M, Ughade S, Bhagat B, Agarwal V, Kulkarni H. Bacterial vaginosis in early pregnancy and adverse pregnancy outcome. *J.Obstet.Gynaecol.Res*. 27:175-81 (2001)
72. Paige DM, Augustyn M, Adih WK, Witter F, Chang J. Bacterial vaginosis and preterm birth: a comprehensive review of the literature. J.*Nurse Midwifery* 43:83-9 (1998)
73. Haefner HK. Current evaluation and management of vulvovaginitis. *Clin.Obstet.Gynecol*. 42:184-95 (1999)
74. Romero R, Manogue KR, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC et al. Infection and labor. IV. Cachectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. *Am.J.Obstet.Gynecol*. ;161:336-41 (1999)
75. Cherouny PH, Pankuch GA, Romero R, Botti JJ, Kuhn DC, Demers LM et al. Neutrophil attractant/activating peptide-1/interleukin-8: association with histologic chorioamnionitis, preterm delivery, and bioactive amniotic fluid leukoattractants. *Am.J.Obstet.Gynecol*. 169:1299-303 (1993)
76. Hillier SL, Witkin SS, Krohn MA, Watts DH, Kiviat NB, Eschenbach DA. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. *Obstet.Gynecol*. 81:941-8 (1993)
77. Fox H. Pathology of the Placenta, 2nd edition. *W.B.Saunders Company LTD* 1997.
78. Okada T, Matsuzaki N, Sawai K, Nobunaga T, Shimoya K, Suzuki K et al. Chorioamnionitis reduces placental endocrine functions: the role of bacterial lipopolysaccharide and superoxide anion. *J.Endocrinol*. 155:401-10 (1997)
79. Denison FC, Kelly RW, Calder AA, Riley SC. Cytokine secretion by human fetal membranes, decidua and placenta at term. *Hum.Reprod*. 13:3560-5 (1998)
80. Fortunato SJ, Menon RP, Swan KF, Menon R. Inflammatory cytokine (interleukins 1, 6 and 8 and tumor necrosis factor-alpha) release from cultured human fetal membranes in response to endotoxic lipopolysaccharide mirrors amniotic fluid concentrations. *Am.J.Obstet.Gynecol*. 174:1855-61 (1996)
81. Menon R, Swan KF, Lyden TW, Rote NS, Fortunato SJ. Expression of inflammatory cytokines (interleukin-1 beta and interleukin-6) in amniochorionic membranes. *Am.J.Obstet.Gynecol*. 172:493-500 (1995)
82. Romero R, Baumann P, Gomez R, Salafia C, Rittenhouse L, Barberio D et al. The relationship between spontaneous rupture of membranes, labor, and microbial invasion of the amniotic cavity and amniotic fluid concentrations of prostaglandins and thromboxane B2 in term pregnancy. *Am.J.Obstet.Gynecol*.168:1654-64 (1993)
83. Hirsch E, Blanchard R, Mehta SP. Differential fetal and maternal contributions to the cytokine milieu in a murine model of infection-induced preterm birth. *Am.J.Obstet.Gynecol*. 180:429-34 (1999)
84. Ando M, Takashima S, Mito T. Endotoxin, cerebral blood flow, amino acids and brain damage in young rabbits. *Brain Dev*. 10:365-70 (1988)
85. Dammann O, Leviton A. Does prepregnancy bacterial vaginosis increase a mother's risk of having a preterm infant with cerebral palsy? *Dev.Med.Child Neurol*. 39:836-40 (1997)
86. Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin- 1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. *Am.J.Obstet.Gynecol*. 177:19-26 (1997)
87. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci.Lett*. 172:151-4 (1994)
88. Hu WH, Johnson H, Shu HB. Tumor necrosis factor-related apoptosis-inducing ligand receptors signal NF-kappaB and JNK activation and apoptosis through distinct pathways. *J.Biol.Chem*. 274:30603-10 (1999)

89. Mogi M, Togari A, Kondo T, Mizuno Y, Komure O, Kuno S et al. Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *J.Neural Transm*. 107:335-41 (2000)
90. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debre P et al. FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, in vitro, production of nitric oxide and tumor necrosis factor-alpha in glial cells. *J.Neurosci*. 19:3440-7 (1999)
91. Adamson GM, Billings RE. Cytokine toxicity and induction of NO synthase activity in cultured mouse hepatocytes. *Toxicol.Appl.Pharmacol*. 119:100-7 (1993)
92. Floyd RA, Hensley K, Jaffery F, Maidt L, Robinson K, Pye Q et al. Increased oxidative stress brought on by pro-inflammatory cytokines in neurodegenerative processes and the protective role of nitrone-based free radical traps. *Life Sci*. 65:1893-9 (1999)
93. Castano A, Herrera AJ, Cano J, Machado A. Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. *J.Neurochem*. 70:1584-92 (1998)
94. Bronstein DM, Perez-Otano I, Sun V, Mullis SS, Chan J, Wu GC et al. Glia-dependent neurotoxicity and neuroprotection in mesencephalic cultures. *Brain Res*. 704:112-6 (1995)
95. Gayle DA, Ling ZD, Tong C, Landers T, Lipton JW, Carvey PM. Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-alpha, interleukin-1beta, and nitric oxide. *Brain Res.Dev.Brain Res*. 133:27-35 (2002)
96. Jarskog LF, Xiao H, Wilkie MB, Lauder JM, Gilmore JH. Cytokine regulation of embryonic rat dopamine and serotonin neuronal survival in vitro. *Int.J.Dev.Neurosci*. 15:711-6 (1997)
97. Kabiersch A, Furukawa H, del Rey A, Besedovsky HO. Administration of interleukin-1 at birth affects dopaminergic neurons in adult mice. *Ann.N.Y.Acad.Sci*. 840:123-7 (1998)
98. Lu X, Bing G, Hagg T. Naloxone prevents microglia-induced degeneration of dopaminergic substantia nigra neurons in adult rats. *Neuroscience* 97:285-91 (2000)
99. Bruce-Keller AJ. Microglial-neuronal interactions in synaptic damage and recovery. *J.Neurosci.Res*. 58:191-201 (1999)
100. Gao HM, Hong JS, Zhang W, Liu B. Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *J Neurosci* 23:1228-36 (2003)
101. Liu B, Wang K, Gao HM, Mandavilli B, Wang JY, Hong JS. Molecular consequences of activated microglia in the brain: overactivation induces apoptosis. *J.Neurochem*. 77:182-9 (2001)
102. Ling ZD, Gayle DA, Lipton JW, Carvey PM. Prenatal Lipopolysaccharide Alters Postnatal Dopamine in The Laboratory Rat. In: Catecholamine Research: From Molecular Insights to Clinical Medicine. T. Nagatsu, T Nabeshima, R. McCarty, D Goldstein Eds. *New York: Kluwer Academic/Plenum Publishers*, 209-12 (2002)

Key Words: Lipopolysaccharide, Cytokine, Dopamine, Parkinson's Disease, Prenatal, Toxicity, Endotoxin, Inflammation, Review

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