2335 Erk ½ Activation Is Evident in Activated Microglia From the Striatum and Substantia Nigra of MPTP-Induced Mouse Models of PD

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Parkinson's disease (PD) is a debilitating, age-related disorder characterized by selective degeneration of dopaminergic neurons in the midbrain substantia nigra (SNc). Dopaminergic neurons originating here project to the striatum. Although studies have suggested that ERK ½ in the brain is activated after MPTP exposure, no study has yet been done to demonstrate whether such activation takes place in neurons or in glia. In the current study, we have utilized both an acute and a repeat dose mouse model of PD using the neurotoxicant MPTP as the causative agent. Immunohistochemical studies using phospho ERK ½ antibody suggest that ERK ½ activation takes place in both the striatum and SNc in both animal models. Moreover, double immunolabeling studies using phospho ERK ½ and CD11b suggest that the phospho ERK ½ is present exclusively in the microglia and not in the astrocytes. Western Blot results suggest that there are no alterations in ERK in either MPTP treated animals or controls; however, phospho ERK ½ was found to be significantly increased both in the striatum and SNc in both mouse PD models. Tyrosine hydroxylase (TH) immunolabeling revealed significant decreases in dopaminergic neurons in the SNc in both animal models concomitant with activation of microglia and ERK activation. This study suggests that ERK activation takes place following MPTP treatment and that activation of ERK is occurring primarily in the microglia. The results of this study also suggest that ERK activation may be involved in transcriptional activation of microglia following neurotoxicant insult. Study supported by approved NCTR protocols E751201 and E747701

2336 MPTP Neurotoxicity Is Highly Concordant Between the Sexes in BXD Recombinant Inbred Mouse Strains

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Continuing our previous work in which we showed wide-ranging strain differences in MPTP neurotoxicity in male mice among ten BXD recombinant inbred strains, we replicated our work in females from nine of the same strains. Mice received a single s.c. injection of 12.5 mg/kg MPTP or saline. Forty-eight hours later the striatum was dissected for neurochemical analysis. Striatal dopamine (DA) and its metabolites, DOPAC and HVA, striatal serotonin (5-HT) and its metabolite, 5-HIAA, were analyzed using HPLC. Tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) were measured using ELISA. There were wide genetic variations in the DA, DOPAC, HVA, TH and GFAP responses to MPTP. We also performed principal component analysis (PCA) on the difference values, saline minus MPTP, for DA, DOPAC, HVA and TH and mapped the dominant principal component to a suggestive QTL on chromosome 1 at the same location that we observed previously for males. Moreover, there were significant correlations between the sexes for the effect of MPTP on DA, HVA, and TH. Our findings suggest that the systems genetic approach as utilized here can help researchers understand the role of sex in individual differences. The same approach can pave the way to understand and pinpoint the genetic bases for individual differences in pathology attributable to toxicants. Such systems genetics approach has broad implications for elucidating gene-environment contributions to neurodegenerative diseases.

2337 Nurr1 Activation Prevents Neurotoxic Injury in the MPTP Model of Parkinson's Disease

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons of the ventral midbrain, associated with inflammatory activation of glial cells. The orphan nuclear receptor Nurr1 (NR4A2) suppresses inflammatory gene expression in glial cells and also positively regulate genes associated with the production/release of dopamine (DA) in neurons. Despite these known functions of Nurr1, an endogenous ligand has yet to be discovered. We previously reported that the phytochemical-based compound, 1,1-bis(3'-indolyl)-1-(p-chlorophenyl) methane (C-DIM12), activates Nurr1 in neural cells, suppresses inflammatory gene expression in primary astrocytes and induces a dopaminergic phenotype in neuronal cultures. In current studies, an *in vivo* approach was undertaken to examine the capacity of C-DIM12 to protect against loss of dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mice were treated (+/-) MPTP (20 mg/Kg) every four days and (+/-) C-DIM12 (25 mg/Kg) every day for 2-weeks. Motor function was monitored during the course of treatment by open field activity and gait analysis. Preliminary data from this study revealed a significant preservation of TH-positive neurons in the substantia nigra (SN), as determined by design-based (3D) stereological methods, as well as preservation of TH protein levels in the striatum, as determined by immunoblotting. C-DIM12 treatment also inhibited the mRNA expression of multiple neuroinflammatory genes in qPCR array studies. To further examine the cell-specific role of Nurr1 in glial cells and DA neurons in the SN, we used adeno-associated viral (AAV) vectors to selectively overexpress Nurr1 in GFAP (+) astrocytes and DA (+) neurons following intracerebroventricular injections in neonates. Expression of Nurr1 was determined by whole-brain image montaging and CLARITY tissue transmutation. These data suggest that Nurr1 is a direct regulator of dopaminergic function in glial cells as well as neurons and that this receptor may be a viable target for selected small molecule therapeutics. This work was supported by a grant from the Michael J. Fox Foundation (RBT) and by NIH-ES021656 (RBT).

2338 Metabolic Flux Analysis in Human Dopaminergic Neurons Under Toxicant Stress

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Background: Luhmes cells are non-transformed conditionally immortalized human neuronal precursor cells which can be differentiated to dopaminergic neurons within six days. They are used as a model for (developmental) neurotoxicity. The cells are suitable to be used for both classical viability analysis and the study of neurite outgrowth. Methods: Either proliferating or differentiated (by addition of cAMP, tetracycline and GDNF) cells were used for experiments. Metabolomics analysis was performed using an LC-MS approach. For isotope labeling metabolic flux analysis cells were fed with 13C-labelled glucose or glutamine. Subsequent GC-MS/MS analysis was used to quantify metabolite pools and fluxes. Flux maps resulted from computational modeling based on absolute concentrations and label incorporations into the CCM-metabolites. Results: Upon induced differentiation, Luhmes cells change their phenotype from precursor to fully differentiated dopaminergic neurons, e.g. shown by cell cycle arrest and expression of tyrosine hydroxylase. This change in phenotype is accompanied by a change in concentrations and fluxes of intermediates of the central carbon metabolism (CCM). By the use of stable isotope labelled metabolite precursors, a flux map of the CCM of Luhmes was established for undifferentiated and differentiated cells. The metabolic flux analysis indicated that precursor cells have a stem cell-like metabolism, whereas differentiated cells acquire a neuronal-like metabolism. Based on this set of background data, the metabolic impact of toxic chemicals can be described in high detail for different neuronal differentiation stages.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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