and in higher doses. 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) is produced in meat during high-temperature cooking, and it bears structural resemblance to dopamine and dopaminergic toxicants. Our previous work determined that PhIP and its Phase I metabolite, N-OH-PhIP, are selectively neurotoxic to dopaminergic neurons in primary midbrain cultures. Treatment of cultures with PhIP and N-OH-PhIP causes neurite degeneration in dopaminergic neurons. More recently, we also determined that neurite lengths in non-dopaminergic neurons were not affected by PhIP treatment, further illustrating selective toxicity to dopaminergic neurons. In this study, we assess PhIP neurotoxicity *in vivo* after systemic administration to male SD rats at 6-7 weeks of age. For acute exposure, we treated rats with a single oral gavage dose at 100 or 200 mg/kg (n=2 per group). Animals were euthanized 8 or 24 h after exposure. Our initial experiments showed almost a two-fold increase in nitrotyrosine levels in SN of 200 mg/kg PhIP-treated rats for 8 h compared to control (n=42-97 neurons/analyzed group). This difference was observed to increase in a time-dependent manner. We also tested subacute toxicity of PhIP. We treated rats with 75 mg/kg PhIP by oral gavage, three times a week for 4 weeks (n=9 per group). PhIP-treated rats showed significantly reduced weight gain after one week, but motor tests (postural instability and rearing tests) failed to show overt differences between treated and control groups. Our future studies will continue to assess neurotoxicity of acute and subacute PhIP administration *in vivo*, including oxidative stress, neurotransmitter levels and DNA damage to determine whether preclinical PD features are reproduced.

PS 1523 TIMP1 mRNA Expression Is a Biomarker of Astrogliosis: Evidence from Multiple Neurotoxicants and BAC-TRAP Technology

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A characteristic feature of neurotoxicity is the selective and unpredictable damage to specific neural cells. This lack of target identity constitutes a substantial barrier to neurotoxicity detection and characterization. Evaluating astrogliosis overcomes this problem as reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Previously, we introduced ALDH1L1 BAC-TRAP (translating ribosome affinity purification) technology for neurotoxicological evaluation as it allows for characterization of the actively translating transcriptome of astrocytes responding to toxicant-induced neural damage. To begin to characterize the astrocyte injury-response transcriptome, ALDH1L1 BAC-TRAP mice were given a single 12.5 mg/kg s.c. dose of MPTP, a well characterized dopaminergic neurotoxicant that induces significant astrogliosis. Striatal tissue (12, 24 and 48 hrs post MPTP) was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. Changes induced by MPTP damage were determined by microarray (Illumina Expression BeadChip) and the dataset interrogated using Ingenuity Pathway Analysis. MPTP induced robust transcriptome changes in genes previously identified as astrocyte specific with an 800-fold increase in TIMP1, a finding suggestive of the role of astrocytes in extracellular matrix remodeling. These data were confirmed by qPCR and extended to two additional neurotoxicants, methamphetamine (METH) and kainate (KA). As with MPTP, METH and KA mRNA expression analyses showed large fold increases in TIMP1. Prior treatment with the stress hormone, corticosterone (CORT) is known to increase astrogliosis and damage after METH and to decrease the same measures after KA. TIMP1 expression followed the same pattern. These data suggest that astrocytes function in extracellular matrix degradation and tissue remodeling following neurotoxic insult.

PS 1524 *In Vitro* 3D Dopaminergic Model to Study (Developmental) Neurotoxicity and Parkinsonism

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The environmental contribution to the increasing number of neurodevelopmental disorders, such as autism, is currently in focus and initiated the development of human relevant *in vitro* models for DNT. Likewise, the development of a relevant *in vitro* model to study Parkinson's neurodegeneration affecting dopaminergic neurons is of high interest. We developed a 3D *in vitro* human model, which can be implemented for both DNT and neurodegeneration research. This model is based on the 3D differentiation of LUHMES progenitor cells into mature dopaminergic neurons within 6-9 days. We have optimized the 3D protocol to control size and homogeneous differentiation. The spheroids can be kept in culture up to 21 days, which is twice longer than traditional 2D cultures. The anti-proliferation drug Taxol was used to reduce proliferation within the aggregates. Treatment with Taxol on day 3-5 led to a decrease in proliferation (58.2 % to 11.3 % Ki-67 positive cells) on day 6, a decrease in spheroid size, an increase in the expression of mature neuronal markers TH, NeuN, β-TubIII and SYN1 as well as neuronal-specific microRNAs. Interestingly, we observed increased neuronal arborization in Taxol-treated cells, which was measured by immunostaining for TH, MAP2 and NF200. Compound penetration and apoptosis in differentiating spheroids were studied over time using confocal imaging. To overcome the known limitations of high content confocal imaging in 3D and optimize the visualization of neuronal morphology, we (i) co-differentiated the wild type LUHMES (98% of the culture) with LUHMES expressing RFP (2% of the culture) and (ii) established an optical clearance method to increase confocal microscope resolution. Thereby, we established an imaging workflow that allows quantification of neurotoxicant effects on neurite outgrowth, branching and synaptogenesis in 3D. Two reference compounds known to induce the parkinsonism (MPP+ and rotenone) was used to demonstrate the suitability of this model for (developmental) neurotoxicological studies.

PS 1525 Altered Optineurin Expression in Cellular and Rodent Models of Parkinson's Disease

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Optineurin(OPTN) is a genetic factor in glaucoma and amyotropic lateral sclerosis. Multiple functional roles have been identified, including in vesicle trafficking, Golgi apparatus organization, induction of macroautophagy and cell cycle. Recent data has shown that OPTN aggregates in vulnerable neurons in several neurodegenerative diseases, including frontotemporal lobar degeneration, Alzheimer's, Huntington's, and Parkinson's disease(PD). Major pathological hallmarks in PD include aggregation of α-synuclein into Lewy bodies and progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN). Macroautophagy is a primary route for degradation of α-synuclein and protein aggregates. This pathway is dysregulated in PD. Currently, data on roles for OPTN in the pathogenesis of PD is very limited. Given OPTN's involvement in many cellular pathways that are also implicated in PD pathogenesis, we characterized basal brain expression and response to DA neurotoxicants. Here we show immunohistochemical evidence that OPTN is enriched in DA neurons in the SN. Using primary rat mesencephalic cultures that contain DA and nonDA neurons and glia, OPTN expression increased after acute exposure to methamphetamine, paraquat, or overexpression of α-synuclein (both mutant and wild type), compared to control, assessed by quantitative immunofluorescence. These data demonstrate that OPTN has high expression in DA neurons, and its expression is increased when PD is modeled by toxicant insult or genetically. Ongoing experiments are examining OPTN expression in SN of rats acutely exposed to paraquat or rotenone, toxicants used to model PD and linked to increased risk. Here, brains have been sampled prior to overt neurodegeneration, representing a 'preclinical' sampling point. Our next steps are to investigate the interaction between OPTN and macroautophagy after PD-relevant insults. Funding: NIEHS/NIH ES019879 (J.R.C.)

PS 1526 Transcriptional Regulation of the Compensatory Signaling Molecule Prokineticin-2 during Neurotoxic Stress in Dopaminergic Neuronal Cells

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While cell signaling mechanisms underlying neurotoxic injury have been actively studied in recent years, signaling molecules contributing to compensatory survival signaling are largely unknown. We recently discovered that a secretory neuropeptide prokineticin-2 (PK2) is upregulated in dopaminergic neurons during early stages of neurotoxic stress in experimental models of Parkinson's disease (PD) and plays a neuroprotective role against neurotoxic stress. In this study, we characterized the transcriptional regulatory mechanisms of PK2 in a dopaminergic neuronal model of PD. *In silico* analysis of PK2 promoter region detected binding sequences for key oxidative stress-related transcription factor, hypoxia-inducible factor (HIF) and the neuronal survival and differentiation factors, early growth response (EGR) proteins. To validate these findings, we cloned the 5'-flanking region (1kb) of the human PK2 gene into a luciferase reporter vector and then transfected into MN9D dopaminergic neuronal cells to study the PK2 gene regulatory mechanism. Treatment with HIF1α activators 3,4-dihydroxybenzoate (DHB) or cobalt significantly increased PK2 promoter activity, implying that HIF1α plays a role in PK2 gene expression. We confirmed this finding by cotransfection studies, wherein overexpression of HIF1α and HIF2, but not inactive HIF1α, stimulated PK2 promoter activity. Furthermore, HIF1α overexpression and DHB treatment synergistically activated the PK2 promoter. Interestingly, EGR and E2F overexpression also enhanced the PK2 promoter activity. To further validate the role of HIF, EGR and E2F in regulating PK2 gene expression, we measured PK2 mRNA and protein

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