INA-12

12th Biennial Meeting of the International Neurotoxicology Association

Gene-Environment Interactions in Neurotoxiciology

June 7-12, 2009

Ma'aleh Hachamisha Conference Center
Judean Hills, Israel

Program and Book of Abstracts

INA-12 website: http://www.neurotoxicology.org
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International Neurotoxicology Association

12th Biennial Meeting

7-12 June 2009
Ma'ale Hachamisha Conference Center
Israel

Dear Conference Attendees,

Welcome to the 12th Biennial Meeting of the International Neurotoxicology Association!

The theme of the INA-12 meeting is "Gene-Environment Interactions in Neurotoxicity." The conference will explore the most recent scientific research regarding the influence of genetic composition on the response of individuals to neurotoxic compounds. In keeping with the tradition of the INA meetings, the conference will begin with a keynote lecture honoring the memory of Dr. Jacob Hoissma. This year we are very fortunate to have the Hoissma Lecture presented by Professor Hermona Soreq, Dean of the Faculty of Science at The Hebrew University in Jerusalem.

Throughout the week, we will have an exciting program of symposia featuring some of world's leading scientists in neurotoxicology and related disciplines, who will explore gene-environment interactions and many other aspects of neurotoxicology. One of the goals of the INA is to recruit and energize young investigators to pursue careers in neurotoxicology, and therefore the program also features a student symposium containing talks from students and postdoctoral fellows who were selected to receive student support awards. The two poster sessions will feature many topics of interest to neurotoxicologists and reflect the depth and breadth of neurotoxicology research underway around the world.

The INA meetings truly reflect an international organization, with attendees coming from dozens of countries worldwide. Occasions to meet and interact with such a collection of scientists are rare, and we hope you will enjoy the event and take full advantage of its opportunities.
We are delighted to have you here.

Sincerely yours,

Yoram Finkelstein, MD, PhD
Chair, Local Organizing Committee

William K. Boyes, PhD
President, INA

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SOCIAL PROGRAM

Sunday, June 7

4:00 – 4:30 PM **Welcome**

6:00 – 7:30 PM **Welcome Dinner**

8:00 – 9:00 PM **An Overview of Israel: History and Archaeology**
Yossi Maimon

……

Monday, June 8

6:00 – 6:45 PM **Dinner**

7:00 PM Promptly **Evening Tour – Western Wall and Tunnels**
Buses will leave at 7:00 for the tour.

……

Tuesday, June 9

6:00 – 7:30 PM **Dinner**

……

Wednesday, June 10

9:00 AM – 5:00 PM **Full Day Tour of Massada and Dead Sea**

Please be certain to bring hat, water, sunscreen and comfortable walking shoes.
If time permits, we may take a dip in the Dead Sea so bring along a towel and bathing suit.

7:30 – 9:00 PM **Gala Banquet**
Greetings: **William Boyes, President, International Association of Neurotoxicology**
Yoram Finkelstein, Conference Chair
Honorary Guest: **Dr. Yossi Inbar, Director General, Ministry of Environmental Protection**

9:00 – 10:00 PM **Shuli Natan, Israeli folk singer**

……

Thursday, June 11

6:30 – 8:00 PM **Dinner**

……

Optional Tours
Friday, June 12 **Tour 1B, Jerusalem, New City**
Departure 12:00
Sunday, June 14 **Tour 2, Caesarea, Haifa and Acre**
Departure 8:00
Monday, June 15 **continuation of Tour 2, Galilee and Jordan Valley**

SCIENTIFIC PROGRAM

Sunday, June 7

4:00 – 4:15 PM

Welcome

4:15 – 5:15 PM

Hooisma Lecture
Prof. Hermona Soreq, Dean, Faculty of Science, Hebrew University of Jerusalem 1
Gene-Environment Interactions in the Mammalian Cholinergic System: Implications for Health and Disease

Monday, June 8

9:00 AM – 12:00 PM

Symposium 1:
Gene-Environment Interactions in Determining Resistance or Susceptibility to Neurodegeneration: What can be Learned from Mammals and Non-Mammals?
Chair: Michael Aschner

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Shared Neurotoxicity Mechanisms in C. Elegans and Mammalian Models

Ed Levin, PhD, Professor, Duke University 3
Developing Zebrafish Methods to Learn How Neurotoxic Events Impact Behavioral Function

Moussa B.H. Youdim Ph.D, Professor Seoul Yonsei University,
Hong Kong PolU and Hong Kong Universities, Professor Emeritus, Technion 4
Novel Therapeutic Approaches Constituting Multimodal Neuroprotective and Neurorestorative Drugs for Parkinson's and Alzheimer's Disease

Cristina Suñol, PhD, Professor, Instituto de Investigaciones Biomédicas de Barcelona 5
Way Forward for Using In Vitro Neurotoxicity Models in Testing Strategy: Update on FP6 Integrated Project "ACUTETOX"

James Olopade, DVM, Ph.D., Professor, University of Ibadan, Nigeria 6
Erythropoietin, Desferroxamine and Tiron are Protective against Vanadium Induced Demyelination and Oxidative Stress in the Rat Brain

1:30 PM – 4:30 PM

Symposium 2:
Gene-Chemical Interactions: Proliferation, Neurogenesis and Differentiation
Chair: Donald Fox

4:30 – 5:30 PM

INA Members Business Meeting

Tuesday, June 9, 2009

9:00 AM – 12:00 PM

Symposium 3:
Novel Signaling in the Expression of Neurotoxicity
Chairs: Anumantha Kanthasamy, Stephen Bondy

1:30 PM – 4:00 PM

Symposium 4:
Endocannabinoid Signaling in Neurotoxicity & Neuroprotection
Chair: Carey Pope

4:00 PM – 5:30 PM

Symposium 5:
Student Symposium
CHAIR: Boyes ???????

7:30 PM – 9:30 PM

Poster Session (Posters are listed at end of program)

Thursday, June 11, 2009

9:00 AM – 12:00 PM

Symposium 6:
Strategy For In Vitro Neurotoxicity and Developmental Neurotoxicity Testing (DNT): Contribution of International Integrated Research Projects
Chairs: Anna Price, Lenas Petros

1:30 PM – 4:30 PM

Symposium 7:
Peaceful Use of Disastrous Neurotoxicants
Chairs: Yoram Finkelstein, Uri Wormser

4:30 PM – 6:30 PM

Symposium 8: Of Exposure and Susceptibility: How Important are Both?
Chairs: Elihu D. Richter, Philippe Grandjean

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Elihu D. Richter, MD, Professor, Hebrew University – Hadassah School of Medicine 32
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Philippe Grandjean, PhD, Professor, Environmental and Occupational Medicine, University of Odense and Harvard University 33
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Poster Session (Posters are listed at end of program)

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9:00 AM – 12:00 PM

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Chair: Carlos Singer

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Genes, Environment and Susceptibility in Parkinson's Disease

Donato DiMonte, PhD, Professor, Director of Basic Research, Parkinson's Institute and Clinical Center 36
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Syed Ali, PhD, Head, Neurochemistry Laboratory, FDA National Center for Toxicological Research 37
Selective Alterations in the Gene Expression, Transcription Factors and Mitochondrial Membrane Proteome in MPTP-Induced Model of Parkinson's Disease

Walter Bradley, MD, Professor, University of Miami 38
Role of a Ubiquitous Neurotoxic Non-Natural Amino Acid, BMAA, and Cyanobacteria as an Environmental Toxin Causing ALS, Alzheimer's Disease and Parkinson's Disease

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Yoram Finkelstein, Conference Chair

POSTERS
In alphabetical order by the first author
The page number on the abstract book corresponds to the number on posterboard)

GENE-ENVIRONMENT INTERACTIONS IN THE MAMMALIAN CHOLINERGIC SYSTEM: IMPLICATIONS TO HEALTH AND DISEASE

Hermona Soreq, PhD

The Hebrew University of Jerusalem, Department of Biological Chemistry;
Safra Campus-Givat Ram; Jerusalem 91904; Israel; soreq@cc.huji.ac.il

Cholinergic neurotransmission notably modulates diverse evolutionarily conserved features such as motor control over breathing and movement, working memory and brain-to-body communication. This is why the cholinergic pathway is targeted by widely used cholinesterase inhibitor drugs and insecticides and why environmental exposure to agricultural cholinesterase insecticides presents a major health problem with yet unclear underlying poisoning mechanisms. Novel therapeutic approaches are being developed which involve the use of engineered recombinant human cholinesterases as decoys. Environmental exposure impairs functioning of the brain, where inhibition-induced changes in acetylcholinesterase *ACHE* gene expression modify its combinatorial series of protein products, with different N- and C-termini due to alternate promoter usage and 3'-alternative splicing. This induces diverse signalling cascades with distinct consequences on the risk of, and rate of progression of Alzheimer's and Parkinson's disease. Carriers of inherited cholinesterase mutants, such as the common and unstable BChE-K variant of the butyrylcholinesterase *BCHE* gene may be particularly sensitive to such exposure. Cholinesterase inhibition further elevates acetylcholine levels, which changes the suppression of peripheral inflammatory responses by macrophages. Recently, we discovered that inflammatory stimuli induce leukocyte over-expression of the AChE-targeting micro-RNA-132, and identified this AChE mRNA-targeting micro-RNA as a functional regulator of the brain-to-body resolution of inflammation. Nucleic acids-based agents targeting specific cholinesterase RNA transcripts can hence open new avenues for study and therapeutic manipulations of environmental exposure to anti-cholinesterases.

SHARED NEUROTOXICITY MECHANISMS IN C. ELEGANS AND MAMMALIAN MODELS

Alexandre Benedetto, PhD, Catherine Au, Michael Aschner, PhD

Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232, USA, Michael.aschner@vanderbilt.edu

Occupational exposure to manganese (Mn) has been implicated in a Parkinsonian-like syndrome, affecting the dopaminergic (DAergic) system. We hypothesized that the commonalities exhibited in both Parkinson's disease (PD) and manganism rely on common molecular pathways. We used C. elegans strains mutated for PD and DAergic circuitry-specific genes and evaluated their sensitivity to Mn exposure. Mn treatments specifically lead to DAergic neuron degeneration, while sparing other neurotransmitter systems, such as GABAergic or cholinergic neurons. We further establish that the dopamine (DA) transporter knock-out dat-1 (ok157) and the DA receptor dop-2(vs105);dop-1(vs100);dop-3(vs106) triple mutant are hypersensitive to Mn exposure. Conversely, the vesicular monoamine transporter 2 cat-1(e1111) and the tyrosine hydroxylase cat-2(e1112) mutants are hyper-resistant. Combined with measurements of DA content in these strains, our results suggest that elevated DA levels sensitize the worm to Mn toxicity. We propose that extracellular rather than intracellular dopamine is involved in Mn toxicity in C. elegans, which would explain the sensitivity of DAergic neurons to Mn exposure. In addition, we have characterized DMT1-like proteins in the nematode C. elegans and explored their role in Mn homeostasis and toxicity in the whole animal. We identified three DMT1-like genes in C. elegans, smf-1, smf-2 and smf-3. We found that all can functionally substitute for loss of their orthologues in the yeast S. cerevisiae. In the nematode, GFP-fusions revealed that SMF-1 and SMF-3 localize to the apical membrane of the gut epithelium, while SMF-2 was detected in the marginal pharyngeal epithelium. smf-1 and smf-3 mutants are resistant to Mn-induced lethality, while smf-2 mutants are hypersensitive. Analysis of metal content upon Mn exposure in smf mutants revealed that SMF-3 is required for Mn uptake, while SMF-2 inhibits it but favors iron (Fe) uptake. Fe levels decreased with increasing Mn exposure in smf-1 and smf-3 mutants. QRT-PCR of smf gene expression showed that high Fe concentrations correlate with accumulation of smf-2 mRNA, supporting a role for SMF-2 in Fe uptake. Finally, SMF-3 was reversibly down-regulated following Mn-exposure. Our work supports a functional conservation for DMT1 orthologues across animals. It suggests that the uptake of Fe and Mn is coordinately regulated. Further, it reveals a complex inter-dependent regulation of DMT1 genes, which explains the Mn-resistant or Mn-sensitive phenotypes of smf mutants. We have therefore defined an in vivo. platform to investigate the role of DMT1-like proteins in metal physiology and toxicology. These data recapitulate many of the known effects of Mn in mammalian systems, and establish C. elegans as a suitable model for studies on genetic pathways involved in Mn toxicity. This project is funded by R01 ES10563 to MA.

DEVELOPING ZEBRAFISH METHODS TO LEARN HOW NEUROTOXIC EVENTS IMPACT BEHAVIORAL FUNCTION

Edward D. Levin, Ph.D.

Department of Psychiatry and Behavioral Sciences, Duke University Medical Center

Zebrafish provide an excellent model for determining the molecular mechanisms of neural function and the impact of neurotoxicants on those neuromolecular processes. The great variety of mutants and the use of morpholinos, which reversibly suppress specific parts of the genome, provide elegant tools for investigating the molecular underpinnings of neurobehavioral function. To complete the spectrum of methods needed to successfully use zebrafish as models of neurobehavioral impairment, valid, reliable and efficient behavioral assessment procedures are needed. Recently, we and others have developed tests of learning and memory, stress response and sensorimotor reactivity for zebrafish, which are sensitive to drug and toxicant effects, similar to those seen in classic rodent assays and humans. Nicotine administered to adult zebrafish improves both learning and memory and has anxiolytic effects in a similar effect as seen in rats and humans. Both the cognitive and anxiolytic effects of nicotine in zebrafish are reversed by nicotinic antagonist treatment. Early developmental exposure to low doses of the organophosphorus pesticide chlorpyrifos impairs learning and potentiates the startle response in zebrafish later in life. Zebrafish models can usefully complement mammalian investigations by providing a high throughput front-end to the investigatory sequence to triage the chemicals for further testing. In addition, molecular tools and reporter techniques for continuous visualization of the developmental process can help determine mechanisms of toxicity. The development of a neurobehavioral test battery for zebrafish will bring these advantages to bear for functional neurotoxicity. Zebrafish can provide useful starting points for the characterization of toxicity with classic mammalian models.

(Support from the Duke Univ Superfund Basic Research Ctr ES010356)

NOVEL THERAPEUTIC APPROACHES CONSTITUTING MULTIMODAL NEUROPROTECTIVE AND NEURORESTORATIVE DRUGS FOR PARKINSON'S AND ALZHEIMER'S DISEASE

Moussa B.H. Youdim

Eve Topf and NPF Centers of Excellence for Neurodegenerative Diseases Research
Technion-Rappaport Family Faculty of Medicine
Haifa, Israel

Discovery of novel multifunctional neuroprotective and neurorestorative drugs has led to new therapeutic approaches for the treatment of Alzheimer's disease (AD) comprise drug candidates designed specifically to act on multiple CNS targets. One major pathology of AD is the accumulation of iron in nucleus bailus, dentate gyrus, amyloid plaques, and tangles. The iron contributes to onset of oxidative stress and glutaminergic excitotoxicity.We have synthesized several multifunctional non-toxic, brain permeable iron chelator drugs, M-30 series, possessing propargyl monoamine oxidase (MAO) inhibitory moiety, with neuroprotective and neurorestorative activities from our prototype iron chelator VK-28. M-30 and its derivatives possess a wide range of pharmacological activities, including pro-survival neurorescue effects, induction of neuronal differentiation and regulation of amyloid precursor protein (APP) and □-amyloid (A□) levels. These drugs reduce apoptosis of SH-SY5Y, PC-12cells and rat cortical cells neuroblastoma cells in a neurorescue serum deprivation model, via reduction of the pro-apoptotic proteins Bad and Bax, and inhibition of the apoptosis-associated phosphorylated H2A.X protein (Ser 139) and caspase 3 activation. In addition they induce the outgrowth of neurites, trigger cell cycle arrest in G0/G1 phase and enhanced the expression of growth associated protein-43, HIF (Hypoxia Inducing Factor) and the neurotrophin, VGEF. This ius associated with the inhibition of iron dependent prolyl-4-hydroxylase that regulates HIF. These compounds have the abiity of converting more than 85% of adult human stem cells in culture into neurons. Furthermore, since APP has been shown to be an iron regulated protein, similar to ferritin, by possessing 5" UTR in its mRNA element, These drugs markedly reduce the levels of cellular APP and □-C-terminal fragment (□-CTF) and A□ peptide in the medium of SH-SY5Y cells and Chinese hamster ovary cells stably transfected with the APP 'Swedish' mutation. As a consequence levels of the non-amyloidogenic soluble APP□ and □-CTF in the medium and cell lysate respectively are increased. These properties and neuroprotective and neurorestorative effects, suggest that these drugs serve as an ideal drug for AD.

WAY FORWARD FOR USING IN VITRO NEUROTOXICITY MODELS IN TESTING STRATEGY: UPDATE ON FP6 INTEGRATED PROJECT "ACUTETOX"

Cristina Suñol[1], Anna Forsby[2]

[1]Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, CIBERESP, Barcelona, Spain (csenqi@iibb.csic.es)
[2]The Arrhenius Laboratories for Natural Sciences, Stockholm University, Stockholm, Sweden

Purpose: To develop a strategy in which general cytotoxicity, together with e.g. organ-specific endpoints and biokinetic features, are taken into consideration in the *in vitro* prediction of oral acute systemic toxicity. This presentation summarizes published results from the ACuteTox Project (Forsby et al., in press; Suñol et al., 2009).

Methods: A set of reference compounds were tested against approximately 50 neuronal endpoints using neural cell lines, and primary neuronal and reaggregate cultures. *In vitro* data were compared with acute human lethal blood concentration (LC50).

Results: The testing of a subset of 20 reference compounds revealed that $GABA_A$ receptor ($GABA_AR$) function, acetylcholine esterase activity, cell membrane potencial (CMP), glucose uptake, total RNA expression and altered gene expression of NF-H, GFAP, MBP, HSP32 and caspase-3 were the best endpoints. Thirty-six additional chemicals were analyzed against this combined battery of tests. No single endpoint was shown to give a perfect improvement in the *in vitro- in vivo* correlation. The functional neuronal endpoints $GABA_AR$ and cell membrane potential measured in primary neuronal cultures and in the SH-SY5Y cell line identified a high number of chemicals as neurotoxic compounds. A combined analysis of NF-H, GFAP, MBP and HSP32 mRNA expression, glucose uptake and total RNA synthesis measured in aggregated embryonic brain cells was the most sensitive assay. Alteration of caspase-3 mRNA expression in cultured cerebellar granule cells gave a good estimate of the LC50 for a small set of referente chemicals.

Conclusions: All outliers in the correlation analysis between basal cytotoxicity and human LC50, except atropine and acetonitrile, were identified as alerts by at least one of the endpoints in the test battery. The genomic biomarkers NF-H, GFAP, MBA, HSP32 and caspase-3 were very sensitive endpoints. They showed the possibilities to use high throughput quantitative microarray analyses for toxicological screening. The combined CMP and $GABA_AR$ assays correctly identified outliers.

Supported by projects: EU FP6-512051, FIS PI06-1212 (Spain),The Animal Welfare Agency and The Foundation for research without animal experiments (Sweden)

ERYTHROPOIETIN, DESFERROXAMINE AND TIRON ARE PROTECTIVE AGAINST VANADIUM INDUCED DEMYELINATION AND OXIDATIVE STRESS IN THE RAT BRAIN

Olopade, James Olukayode*[1,3]. Ponnoru Padmavathi*., Todorich, Bozho*., Mairuae, Nootchanat*., Neely, Beth*., Sirén, Anna-Leena[2], Connor James.*

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[1] Department of Veterinary Anatomy, University of Ibadan, Nigeria
[2] Department of Neurosurgery, University of Würzburg, Germany
[3]Corresponding author: jo.olopade@mail.ui.edu.ng

Purpose: There have been recent dramatic increases in the combustion of fossil fuel and the release of finely particulate vanadium compounds in the Arabian Gulf and Nigeria's Niger Delta. Hypomyelination and/or myelin destruction from lipid peroxidation has been implicated in part to be responsible for the phenotypic expressions of neuromuscular and behavioral deficits seen in vanadium toxicosis. In this study we assessed novel cellular changes caused by vanadium in culture cells and the neuroprotective effects of desferroxamine (DFO), tiron and erythropoietin (EPO) on vanadium induced astrogliosis and demyelination.

Methods: The generation of reactive oxygen species (ROS), cellular hypoxia and erythropoietin expression by vanadium on treated primary astrocyte cultures (150uM sodium metavanadate for 6 hours) was determined using the dichlorofluoroscein (DCF) assay, and western blot (WB) using HIF-1α and EPO antibodies respectively. We also treated 15 day old Sprague Dawley rats with 3mg/kg sodium metavanadate for seven days; three additional groups were similarly treated but were given DFO (300mg/kg/day), tiron (606mg/kg/day) or EPO (5,000U/kg/day) as antidotes concurrently for six days, with a last group as control. Astrocytic reactivity was observed in the corpus callosum using GFAP immunohistochemistry while myelin basic protein (MBP) was quantified through WB.

Results: There was a significant increase in ROS generation in vanadate treated primary astrocyte cultures compared to controls including up regulation in HIF-1α and EPO expressions as seen by WB. There was also a significant increase in GFAP immunoreactivity in the corpus callosum and a decrease in MBP expression in brains of vanadium treated rats compared to controls; these observations *were* markedly attenuated in rats receiving the antidotes.

Conclusion: This work provides novel information that vanadate treatment induces expression of EPO in astrocyte cell cultures, and that antidotes to vanadium via the systemic route can prevent CNS induced demyelination and astrogliosis.

GESTATIONAL LEAD EXPOSURE (GLE) INCREASES THE NUMBER OF LATE-BORN MURINE ROD PHOTORECEPTOR AND BIPOLAR CELLS, BUT NOT MULLER GLIAL CELLS: CELLULAR AND MOLECULAR MECHANISMS

Fox DA[1], Xiao W[1], Johnson JE[1], Hamilton R[1], Chaney S[1], Swaroop A[2], Giddabasappa A[1]

[1]University of Houston, Houston, TX
[2]University of Michigan, Ann Arbor, MI

Early postnatal lead exposure decreases the amplitude of the rod-mediated (scotopic) electroretinogram (sERG) a-wave and b-wave in developing children and experimental animals and produces rod-selective apoptosis. In marked contrast, low-level GLE (8.5-14 μg/dl median blood Pb) increases the amplitude of the sERG in children and rats. To determine the molecular mechanism underlying these novel supernormal sERGs, we also established and validated a murine dose-response model of low-level GLE. Adult central retinas from control and GLE mice were immunolabeled with molecular markers for different retinal cells. Retinal immunoblots, with the same molecular markers, were run. To assess kinetics of retinal cell proliferation and death, developing central and peripheral retinas were immunolabeled for cell cycle markers (BrdU & phosphohistone 3 [PH3]) and apoptotic cells (TUNEL). All retinal sections were examined by light or laser confocal microscopy and stereological techniques were used to determine cell counts. Affymetrix GeneChip® Mouse Genome 430 2.0 Array experiments assessed mRNA levels in developing control and GLE mice retinas. In adult mice, GLE produced a dose-dependent selective increase in the number of late-born rods and bipolar cells. The number of early-born horizontal, amacrine and ganglion cells and late-born Müller glial cells were unchanged. Immunoblots confirmed these results. In central and peripheral retinas from GLE mice, the number of BrdU- and PH3-positive cells increased, retinal progenitor cell (RPC) proliferation was prolonged, and developmental apoptosis decreased. Microarray data analysis followed by real-time PCR and functional classification revealed that genes associated with cell cycle and cell fate determination were up-regulated in GLE mice. Thus, GLE in mice produced a novel phenotype characterized by an increased proliferation and genesis of late-born neurons. The selective and persistent increase of these neurons likely produces the lead-induced supernormal sERGs. Upregulation of cell growth genes appears to underlie the increased and prolonged RPC proliferation and rod and bipolar cell neurogenesis. These findings have relevance for retinal and brain development as well as the permanent cognitive, behavioral and retinal functional alterations observed in GLE children.

NIH Grants ES012482, EY007551, EY007024, EY11115 and Sramek Foundation

ACTIVATION OF THE AH RECEPTOR BY DIOXIN IMPACTS PROLIFERATION AND DIFFERENTIATION OF NEURAL PRECURSOR CELLS

L.A. Opanashuk

Department of Environmental Medicine, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

The ubiquitous and persistent environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been linked to developmental neurotoxicity in humans and experimental animals. TCDD, the most potent dioxin, mediates toxicity via binding to the aryl hydrocarbon receptor (AhR), a ligand-activated member of the bHLH/PAS transcription factor superfamily. These transcription factors serve as environmental sensors and transducers of physiological signals, particularly during development. The endogenous functions of AhR remain unknown. Certain bHLH/PAS proteins have been implicated in the regulation of cell fate determination, proliferation, and differentiation during neurogenesis. Therefore, it is conceivable that AhR plays similar roles. TCDD, through its high affinity binding and activation of the AhR, causes numerous biochemical and pathological abnormalities, particularly following developmental exposure. Deficits in cognitive function, locomotor development, and sexual behavior are some of the most sensitive endpoints associated with perinatal exposure to dioxin-like chemicals. However, the regional, cellular, and gene targets of AhR-mediated TCDD neurotoxicity remain unclear. Our laboratory has determined that cerebellar granule neuron precursors (GNPs) express high levels of transcriptionally active AhR during a critical period of neurogenesis. Moreover, our studies indicate that AhR is expressed by pluripotent neuroepithelial stem cells (NSCs). Observations that these precursor cells are important sites of action for AhR-mediated TCDD neurotoxicity will be discussed. Our studies demonstrate striking effects of TCDD on GNP proliferation, early differentiation, and survival as well as on gene expression patterns associated with those processes. Neurogenesis is also abnormal in $AhR^{-/-}$ mice. These data suggest that TCDD disrupts a normal physiological role of AhR, which compromises proper GNP maturation and ultimately leads to fewer cells in the cerebellum. TCDD also reduces NSC production in the adult hippocampus. Therefore, our data suggest that TCCD exposure might be a potential risk factor for neurodevelopmental disabilities and neurological disorders associated with both abnormal motor and cognitive function by disrupting neurogenesis.

POSTNATAL LEAD EXPOSURE REDUCES HIPPOCAMPAL NEUROGENESIS AND INCREASES THE NUMBER OF PSA-NCAM EXPRESSING CELLS IN THE DENTATE GYRUS OF ADULT RAT BRAIN

A. Zharkovsky

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Purpose. Lead is still widely distributed in the environment, and the consequences of chronic exposure to low levels of lead in childhood have been a matter for extensive research during recent years. All current evidence suggests that there is no threshold below which lead remained without effect. Hippocampus plays an important role in learning and memory and several studies indicate that lead can induce alterations in the hippocampal plasticity. In the present study we aimed to investigate whether low-level lead exposure, during the extended post-natal period, would induce deviations in hippocampus-dependent learning and affect the hippocampal neurogenesis in adulthood.

Methods. Wistar rat pups were exposed to 0.2% lead acetate from postnatal day (PND) 1 to PND 30. Behavioral testing was performed on PND 60 (anxiety testing) and PND 80 (contextual fear conditioning). To assess neurogenesis, bromodeoxyuridine (BrdU) was administered in a dose of 300 mg/kg, i.p. on PND 80. Rats were killed 24 hours (proliferation study) or 3 weeks (survival7differentiation study) after administration of BrdU. The brain sections were processed for the immunohistochemical detection BrdU label (peroxidase method), or fluorescence double immunohistochemistry for BrdU and neuronal or glial markers.

Results. Lead exposed rats demonstrated increased anxiety and impaired contextual fear conditioning. Developmental lead exposure reduced generation of new cells in the dentate gyrus and reduced differentiation of BrdU-positive cells into mature neurons expressing the marker for mature neurons, calbindin. In contrast, the proportions of young not fully differentiated neurons and proportions of astroglial cells, generated from newly born cells, were increased in lead-exposed animals. In the dentate gyrus of control rats, a majority of the newly generated cells expressed phosphorylated CREB. In contrast, in the lead treated rats, the expression of pCREB in BrdU-positive cells was reduced. Further analysis showed that lead exposed rats had increased expression of polysialic acid linked neural call adhesion molecule (PSA-NCAM) in the dentate gyrus. It is known that migration of the newly born cells within the dentate gyrus occurs in the environment provided by PSA-NCAM. Thus, increased expression of PSA-NCAM can facilitate migration of the new cells and their ectopic positioning within the hippocampal formation.

Conclusions. Our data demonstrate that lead exposure during extended postnatal period induces profound alterations in hippocampal neurogenesis in adulthood.

THE COMBINATION OF AGE, GENDER, AND ENVIRONMENTAL ENDOCRINE DISRUPTORS PRESENTS A TANGLED PUZZLE FOR THE PROCESS OF NEUROGENESIS

Bernard Weiss

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Many neurobehavioral functions wane with aging. The process may be accelerated, or the loss of function exacerbated, by exposure to neurotoxicants. Metals such as lead, manganese and methylmercury, pesticides such as parathion and paraquat, and industrial chemicals such as PCBs can diminish functional capacities in aging populations. Declines in neurobehavioral function arise from structural and functional changes in the aging brain, as seen in extreme cases such as Parkinson's disease and Alzheimer's disease. Some of the more common, less dramatic, accompaniments of aging, such as impaired memory, may arise from a diminished capacity for neurogenesis and synaptogenesis. These processes are known to depend, in part, on endocrine mechanisms, particularly the gonadal hormones estrogen and testosterone. Their roles in neurogenesis, in fact, were among the sources of data demonstrating that the process endured beyond early development. Gonadal hormone levels also wane with aging, and we now know that supplemental doses can enhance both neurogenesis and synaptogenesis. It has also become apparent that some of the chemicals labeled as environmental endocrine disruptors (EEDs) have the potential to interfere with the action of gonadal hormones on neurobehavioral function. For example, the plasticizer Bisphenol A, although classified as an estrogenic agent, has the unexpected capacity to eliminate the synaptogenic response elicited by estradiol, and does so at levels below those regarded as free of adverse effects by conventional assessment methods. Because of the role of diminished endocrine function in neurodegeneratve disease, EEDs must be regarded as threats to the capabilities of the aging brain.

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INTERACTION OF ALPHA-SYNUCLEIN WITH PROTEIN KINASE C SIGNALING IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

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Epidemiological and case control studies have shown a strong association between neurotoxic chemical exposure and the development of idiopathic Parkinson's disease (PD). However, understanding cellular and molecular mechanisms of the etiopathogenesis and nigrostriatal degeneration in PD is a formidable challenge. Recently, we uncovered a novel apoptotic pathway involving caspase-3-dependent proteolytic cleavage of a protein kinase C isoform PKCδ that mediates apoptosis in cell culture models of PD following exposure to the organochlorine pesticide dieldrin or the neurotoxic metal manganese. Exposure to these agents resulted in activation of the mitochondrial-dependent apoptotic cascade, starting from cytochrome C release to caspase-3 activation, in a time- and dose-dependent manner. Interestingly, environmental toxicants induced caspase-3-dependent proteolytic cleavage of native PKCδ into a 41 kDa catalytic subunit and a 38 kDa regulatory subunit to persistently activate the kinase. The classic Parkinsonian toxin MPTP also induced proteolytic activation of PKCδ. Additionally, the PKCδ specific inhibitor rottlerin almost completely blocked toxin-induced apoptosis. A novel PKCδ cleavage site specific inhibitor also protected neurotoxicant-induced nigral degeneration. Importantly, overexpression of the kinase-inactive PKCδ^{K376R} mutant, PKCδ-CRM, or PKCδ-siRNA protected against dopaminergic toxin-induced apoptotic cell death, confirming the proapoptotic function of PKCδ in dopaminergic neurodegeneration. Recently, we found that overexpression of human α-synuclein in dopaminergic neuronal cells suppresses proapoptotic PKCδ in an isoform specific protein. Further mechanistic studies revealed that α-synuclein regulate the expression of PKCδ via the NF-kB signaling pathway. This talk will describe some novel findings pertaining to the interaction of PKCδ with alpha-synuclein and the proapoptotic function of PKCδ in neurotoxicity models of Parkinson's disease.

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APLP1 SIGNALING, ALZHEIMER'S PATHOLOGY AND α-SYNUCLEIN AGGREGATION IN THE FRONTAL CORTEX OF MANGANESE-EXPOSED NON-HUMAN PRIMATES

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Chronic manganese (Mn) exposure produces a neurological syndrome with psychiatric, cognitive and parkinsonian features. Gene expression studies in the frontal cortex of *Cynomolgus macaques* exposed to different doses of Mn showed gene expression changes associated with cell cycle regulation, DNA repair, apoptosis, ubiquitin-proteasome system, protein folding, cholesterol homeostasis, axonal/vesicular transport and inflammation. Amyloid-beta (A-beta) precursor-like protein 1 (APLP1), a member of the amyloid precursor family, was the most highly up-regulated gene. Immunohistochemistry confirmed increased APLP1 expression and revealed the presence of A-beta diffuse plaques. Cortical neurons and white matter fibers from Mn-exposed animals exhibited accumulation of silver grains indicative of on-going degeneration. Cortical neurons also expressed nuclear hypertrophy, intracytoplasmic vacuoles, and apoptotis stigmata. The levels of p53 were increased in neurons and glial cells in Mn-exposed tissue. Analysis of another amyloidogenic protein, α-synuclein, also exhibited aggregation in the gray and white matter from Mn-exposed animals. In summary, chronic Mn exposure in non-human primates produces a cellular stress response leading to neurodegenerative changes, diffuse A-beta plaques and α-synuclein aggregation in the frontal cortex. These changes may help explain the cognitive and working memory deficits expressed by these animals

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ALUMINUM, INFLAMMATION AND BRAIN AGING

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Purpose: Aluminum (Al) is the third abundant element in the earth's crust. Al salts are used as food additives in many products. Epidemiological studies have shown that there is a strong relationship between aluminum and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (AD and PD). While normal brain aging is associated with evidence of elevated levels of inflammation even in the absence of extrinsic provocative factors, this is further exacerbated in AD, PD and other age-related neurological disorders. Furthermore, there is good evidence that a causal relation exists between inflammation and the development of neurological deficits. For these reasons, the effect of extended low level exposure to aluminum salts upon indices of brain inflammation was investigated.

Methods: Mice were exposed to aluminum lactate (0, 0.01mM or 0.1 mM) in drinking water for 3 months. The activation of various transcription factors was determined using gel shift mobility assay, proteins were measured using Western blotting, and gene expression was assayed by quantitative PCR of selected mRNAs using a LightCycler instrument. Immunohistochemical procedures utilized a Nikon TE-2000 microscope.

Results: There was a dose-dependent relationship between Al exposure and levels of astroglial and microglial activation in the brain. Increases of inflammatory cytokines TNF-α and IL-1 α were also observed. Gene expression of TNF-α as judged by quantitative analysis of mRNA content, was increased as Al dose increased. Other evidence of oxidative and inflammatory events was apparent as the concentration of Al was increased. These included elevated levels of nitric oxide synthetase, lipid peroxidation and amyloid precursor protein, the latter underscoring the relevance of these changes to AD.

Conclusion: These experimental results suggest that extended exposure to low levels of Al, paralleling those found in some drinking water supplies, may enhance intrinsic levels of cerebral inflammation. Any factor that chronically augments the age-associated elevation of basal cortical inflammation, whether by trauma-related physical means or by biochemical means, is likely to increase the incidence of age-related neurodegenerative diseases. Aluminum is a good candidate for such an undesirable role and the hazard posed by its pervasiveness should not be underestimated.

MECHANISMS OF NEUROBEHAVIORAL TERATOGENICITY: REVERSAL WITH NEURAL PROGENITORS AND OTHER THERAPIES

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The developing septohippocampal cholinergic innervation appears to be particularly susceptible to disruption by a wide variety drugs and chemicals. Thus, prenatal exposure to diverse substances such as heroin, phenobarbital, nicotine and organophosphate pesticides all evoke septohippocampal-related behavioral deficits involving cholinergic failure at the level of receptor-mediated translocation/activation of PKC; in turn, the signaling deficit evokes presynaptic hyperactivity and postsynaptic receptor upregulation that nevertheless fails to compensate for the underlying deficit. Identifying these mechanisms enabled us to design therapies to reverse the impairment in adulthood: a) manipulation of the A10 regulating neural pathways, b) neural grafting of differentiated cholinergic neurons, c) nicotine administration, and most recently, d) transplantation of neural progenitors. We found that neural progenitors exert their therapeutic effect by evoking proliferation of endogenous neural precursor cells that then restore damaged neural circuits to achieve recovery of both synaptic function and behavior; this likely involves a change in the microenvironment in the host brain through the release of cytokines. Currently we are attempting to enhance the potential application of the neural progenitor approach by reducing or eliminating immunological rejection, by autologous transplantation of adult neural stem cells, and by developing a less invasive method of administration, namely administration via the circulatory system.

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THE ENDOCANNABINOID SYSTEM – AN OVERVIEW

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With the identification of the major psychoactive constituent of hashish in 1964 - Δ9-tetrahydrocannabinol - , the identification and cloning of the G-protein-coupled cannabinoid CB1 and CB2 receptors in the late 1980's and early 1990's and the isolation and characterization of their endogenous agonists - the endocannabinoids anandamide and 2-AG - in 1992 and 1995, the cannabinoid system became a central area of investigation in the biological sciences.

A large volume of data points out that endocannabinoids and endocannabinoid-like molecules are part of numerous protective systems, that work in conjunction with the immune system and with various other physiological systems. This is not unexpected as it can be well assumed that in parallel to the highly developed immune system, whose main role is to guard against protein attack, the mammalian body has also developed, by evolution, analogous biological protective systems, against non-protein attacks and metabolic damages.

I intend to present an overview on the chemical biology of the endocannabinoid system and the numerous possible pathways to novel types of therapies.

DIFFERENTIAL ROLE OF ENDOCANNABINOID SIGNALING IN DRUG ABUSE

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Purpose. Addictive drugs exert their behavioral and reinforcing effects through actions on multiple neurotransmitter systems including monoamines, endogenous opioids, and both excitatory and inhibitory amino acids. A growing body of literature also points to an involvement of the endogenous cannabinoid (EC) system in the etiology of drug addiction. However, little is known of the mechanisms through which ECs modulate drug-induced behaviors and there is even less understanding of the effects of prolonged drug exposure on EC signaling. We have developed an *in vivo* microdialysis method that allows the sampling of ECs from the brain interstitial space of free-moving rats and mice. Using this approach we have begun to characterize the effects of various abused substances (alcohol, opiates, psychostimulants) on EC levels in various reward-related brain regions.

Methods. We have used *in vivo* microdialysis to evaluate the effects of voluntary drug self-administration on brain EC levels in rats, and have compared effects produced by different drug classes. We have also characterized alterations in EC signaling associated with alcohol dependence and withdrawal. Using pharmacological manipulations we have evaluated the relative influence of brain EC signaling on the motivation for drug consumption in both non-dependent and drug-dependent animals.

Results. Our data demonstrate that voluntary drug self-administration alters brain EC levels in a dose-dependent and drug-specific manner. Additionally, drug intake appears to produce region specific alterations in EC levels and there is a regionally selective CB_1 receptor influence on drug self-administration behavior. Alcohol dependence is associated with deficient EC signaling in the amygdala and this may contribute to heightened stress sensitivity and excessive alcohol intake.

Conclusions. Brain EC signaling is differentially altered by various abused drugs, and long-term drug consumption may dysregulate EC signaling in a manner that contributes to the maintenance of dependence. These findings indicate the brain EC system may be a viable pharmacotherapeutic target for some types of drug addiction.

ENDOCANNABINOID SIGNALING AND ANTICHOLINESTERASE TOXICITY

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The primary psychoactive component of *Cannabis sativa*, ∆9-tetrahydrocannabinol, alters neurological function by activating specific G protein-coupled cannabinoid receptors. Endogenous ligands for these receptors (endocannabinoids, e.g., anandamide, 2-arachidonyl glycerol) have been identified. In the central nervous system, the cannabinoid type 1 (CB1) receptor primarily mediates the actions of both natural cannabinoids and endocannabinoids (eCBs) by reducing the release of various neurotransmitters *via* a retrograde signaling pathway. In essence, postsynaptic neuron depolarization leads to transient increase in intracellular calcium and stimulation of the synthesis and release of eCBs, which diffuse to the presynaptic terminal to modulate transmitter release. Moreover, some $G_{q/11}$ receptors (e.g., mGluR5, muscarinic M1) are directly coupled to synthesis and release of eCBs. We hypothesized that eCB signaling might modulate cholinergic toxicity elicited by acetylcholinesterase inhibitors by inhibiting release of acetylcholine. Direct and indirect cannabinomimetics reduced the neurotoxicity elicited by both paraoxon and diisopropylfluorophosphate (DFP). Chlorpyrifos and parathion can elicit markedly different degrees of toxicity in rats in the presence of similar acetylcholinesterase inhibition. Under such conditions, chlorpyrifos increased 2-arachidonyl glycerol in hippocampal microdialysates, while parathion had no effect. CB1 knockout mice showed increased acute sensitivity to chlorpyrifos compared to wildtypes. Furthermore, URB597 (an inhibitor of anadamide degradation) partially reversed persistent neurobehavioral changes following acute DFP intoxication. Together, these studies suggest that eCB signaling plays a role in the expression of anticholinesterase toxicity and that targeting this signaling pathway may be therapeutically beneficial.

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DUAL EFFECTS OF LINDANE (γ-HEXACHLOROCYCLOHEXANE) ON CALCIUM HOMEOSTASIS AND EXOCYTOSIS IN RAT PC12 CELLS

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Purpose: Persistent organochlorine pesticides, like lindane, are still abundant in the environment and often found in human and animal tissue samples. Lindane has previously been shown to induce a wide range of adverse health effects, including neurobehavioral effects[1]. These adverse effects are likely mediated, at least partly, via the lindane-induced inhibition of postsynaptic $GABA_A$ and glycine receptors. However, lindane-induced increases in the basal intracellular calcium concentration ($[Ca^{2+}]_i$) have been reported as well[2]. As calcium is the trigger for many cellular processes, including apoptosis, gene expression and exocytosis, we investigated whether lindane affects calcium homeostasis and exocytosis in neuroendocrine PC12 cells.

Methods: Exocytosis was recorded using amperometry[3] and changes in $[Ca^{2+}]_i$ were determined using the calcium-sensitive fluorescent ratio dye Fura-2 in PC12 cells. Mechanisms underlying changes in $[Ca^{2+}]_i$ were investigated using specific voltage-gated calcium channel (VGCC) blockers.

Results: Amperometric recordings demonstrated that lindane (≥10 μM) rapidly induced exocytosis in PC12 cells. Subsequent experiments demonstrated that lindane-induced exocytosis was paralleled by a dose-dependent increase in $[Ca^{2+}]_i$. Using calcium-free medium it was shown that the increase in basal $[Ca^{2+}]_i$ depended largely on extracellular calcium. It was further shown that blocking L-type VGCCs did not prevent the increase in basal $[Ca^{2+}]_i$, which appeared mainly mediated by N-, and P/Q-type VGCCs.

In addition to increasing basal exocytosis and $[Ca^{2+}]_i$, lindane (≥ 10 μM) dose-dependently inhibited depolarization-evoked exocytosis. As expected, this coincided with lindane-induced inhibition of the depolarization-evoked increase in $[Ca^{2+}]_i$. However, in contrast to the increase in basal $[Ca^{2+}]_i$, lindane-induced effects on depolarization-evoked $[Ca^{2+}]_i$ appeared VGCC a-specific.

Conclusions: Lindane induces differential effects on basal and depolarization-evoked $[Ca^{2+}]_i$, resulting in parallel effects on exocytosis. Considering the general nature of these effects as well as the threshold concentration (≥ 10 μM), it can be concluded that the adverse health effects of lindane exposure are not uniquely due to postsynaptic effects but that presynaptic effects play a role as well.

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MOLECULAR MECHANISMS OF COGNITIVE IMPAIRMENT IN PRENATAL NICOTINE EXPOSURE

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Purpose: Prenatal nicotine exposure (Maternal smoking) causes impairment of cognitive process in younger children. It has been shown that hippocampal dependent memory forms are impaired in prenatal nicotine exposed animal models. The AMPA subtype of glutamate receptors play a significant role in synaptic plasticity mechanisms that are required for learning and memory in mammalian hippocampus. Therefore, we investigated the cognitive deficits associated with prenatal nicotine exposure utilizing behavioral studies and the alterations in AMPA receptor mediated synaptic transmission at regional, cellular and molecular levels using electrophysiological methodology. In addition, expression of specific proteins involved in synaptic signaling was evaluated to dissect the role of AMPA receptor mediated synaptic transmission in cognitive impairment in prenatal nicotine exposure.

Methods: Nicotine (6 mg/kg/day) was administered to rats via osmotic mini pumps implanted subcutaneously throughout gestation from the day 3 of pregnancy until pubs were born. Performance of prenatal nicotine and control rats (2-4 weeks) on Y maze task was investigated to assess hippocampus dependent spatial memory deficits. The basal synaptic transmission and longterm potentiation (LTP) in hippocampal Shaffer collateral-CA1 synapse and AMPA receptor mediated synaptic currents were evaluated. We also investigated the whole cell synaptic currents from CA1 pyramidal neurons and the single channel properties of synaptosomal AMPA receptors in control and prenatal exposed groups. The expression of pre synaptic marker Synaptophysin (SYP), post synaptic marker PSD-95, AMPA receptor subunit GluR1 and glutamate vesicle transporter VGLUT1 were investigated by western blotting.

Results: Prenatal exposed rats had fewer visits and spent fewer time in the novel arm in Y maze task suggesting deficit in spatial memory. Basal synaptic transmission including amplitude and frequency of AMPA receptor mediated whole cell synaptic currents from CA1 pyramidal neurons and LTP were significantly decreased in prenatal exposed rats compared to control rats. The single channel properties of synaptosomal AMPA receptors such as channel open probability and mean open time were significantly decreased and the expression of GluR1, SYP, PSD-95 and VGLUT1 also significantly reduced in prenatal nicotine treated group compared to control group.

Conclusion: We conclude that impaired expression and function of AMPA glutamate receptors and associated proteins in maternal smoking resulted in cognitive deficits in younger children.

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DIOXIN INTERFERES WITH NEURAL STEM CELL MATURATION: UNCOVERING POTENTIAL ROLES FOR THE ARYL HYDROCARBON RECEPTOR DURING NEUROGENESIS

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Purpose: Neurogenesis is a multi-step process that includes proliferation and differentiation of neural stem cells (NSCs). Pluripotent NSCs abundantly express the aryl hydrocarbon receptor (AhR), a ligand activated bHLH/PAS transcription factor, which mediates dioxin toxicity. Therefore, we hypothesized that dioxin exposure impedes AhR-mediated signaling events during NSC maturation.

Methods: In this study, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a widespread environmental toxicant and a potent AhR ligand, was used to test this hypothesis. We determined that the C17.2 cell line contained abundant AhR levels and exhibited a multipotent NSC-like phenotype, as characterized by the expression of nestin, a NSC marker.

Results: Our studies revealed that TCDD exposure suppressed NSC proliferation by interfering with the G1 to S cell cycle transition. Furthermore, under defined culture media conditions, we determined that C17.2 cells adopted a neuronal fate, as evidenced by reduced nestin expression, elevated ßIII-tubulin levels and absence of glial markers. AhR protein levels declined but remained detectable following NSC differentiation, suggesting a role for this receptor during NSC maturation to a neuronal lineage. When C17.2 cells were cultured in TCDD under non-depolarizing conditions, AhR expression was not downregulated, as would be expected. Additionally, AhR remained in the nucleus and the expression patterns of nestin, ßIII-tubulin and GFAP proteins were consistent with reduced neural differentiation in response to TCDD. In contrast, when C17.2 cells were exposed to TCDD in depolarizing conditions, AhR expression was downregulated and NSC differentiation was not altered. Our data suggest that AhR activation by TCDD disrupted early NSC differentiation into neurons but that TCDD had distinct actions during activity dependent maturation.

Conclusions: These observations suggest that the inappropriate or sustained activation of AhR by TCDD during neurogenesis can interfere with multiple signaling pathways that regulate NSC proliferation and differentiation, which could adversely impact final cell number in the brain and lead to functional impairments.

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ATTENUATION OF ARSENIC NEUROTOXICITY BY CURCUMIN IN RATS

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Presence of arsenic as a contaminant in ground water in some regions in India and several other countries is a serious risk to humans. Use of arsenic in industries and mining has significantly contributed to enhance its levels in the environment. In view of the continued exposure to arsenic and associated human health risk including neurotoxicity, studies have been carried out to investigate neuroprotective efficacy of curcumin. Rats treated with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) exhibited a significant decrease in locomotor activity (distance traveled 48%, time movement 43%), grip strength (26%) and rota-rod performance (82%) in comparison to controls. A decrease in the binding of 3H-Spiperone to striatal membrane (32%), known to label dopamine – D2 receptors and tyrosine hydroxylase (TH) immunoreactivity in striatum was observed in arsenic treated rats. An increase in arsenic levels in frontal cortex (6.3 fold), corpus striatum (6.5 fold) and hippocampus (7.0 fold) was observed in rats treated with sodium arsenite compared to controls. Exposure to sodium arsenite in rats caused an increase in the level of lipid peroxidation and protein carbonyl and a decrease in reduced glutathione in frontal cortex, corpus striatum and hippocampus as compared to controls. Activity of superoxide dismutase, catalase and glutathione peroxidase was found decreased in these brain regions in arsenic treated rats suggesting increased oxidative stress. Simultaneous treatment with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) and curcumin (100 mg/kg, p.o., 28 days) in rats caused an increase in locomotor activity (distance traveled 38%, time movement 32%), grip strength (20%) and improved the rota-rod performance (76%) as compared to those treated with arsenic alone. An increase in the binding of striatal dopamine receptors (17%) and TH expression was also observed in these rats as compared to those treated with arsenic alone. Arsenic levels in frontal cortex (2.3 fold), corpus striatum (1.2 fold) and hippocampus (1.6 fold) were found to be decreased in rats simultaneously treated with arsenic and curcumin. A decrease in the levels of lipid peroxidation and protein carbonyl and an increase in reduced glutathione levels were observed in corpus striatum, frontal cortex and hippocampus in these rats as compared to those treated with arsenic alone. Interestingly, activity of superoxide dismutase, catalase and glutathione peroxidase was found to be increased in rats treated with arsenic and curcumin simultaneously. No significant effect on any of the above parameters was observed in rats treated with curcumin as compared to controls. A significant protection in behavioral, biochemical and immunohistochemical parameters in rats simultaneously treated with arsenic and curcumin, observed in the present study suggest the neuroprotective efficacy of curcumin.

LONG-TERM EXPOSURE TO DIELDRIN PREVENTS GLUTAMATE TOXICITY BY REDUCING NMDAR AND mGLUR5 FUNCTIONALITY IN PRIMARY CULTURES OF CORTICAL NEURONS

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Purpose: Dieldrin is a previously used pesticide that accumulates in the adipose tissue and the brain of mammals. Despite it is known that an acute exposure to dieldrin evokes convulsions, due to its antagonism on the $GABA_A$ receptor, little is known about the effects of a chronic exposure to this pollutant. We have previously reported that long-term exposure to dieldrin reduces the number of functional NMDA receptors in cerebellar granule cells. In the present work we use primary cultures of cortical neurons, mainly composed of GABAergic neurons, to support these observations in a different neuronal population and to further deep into the mechanisms involved in the toxic action of this pesticide.

Methods: Cell viability: MTT and LDH assays. Functionality of the glutamate receptors (GluR): by measuring the $[Ca^{2+}]_i$ with the fluorescence probe Fluo-3AM. Levels and localization of GluR: Western Blot and immunocytochemistry.

Results: We have observed that the long-term (2 and 6DIV) exposure to a subcitotoxic concentration (60nM) of dieldrin prevents the excitotoxicity and reduces the $[Ca^{2+}]_i$ increase caused by glutamate. However only after 6 DIV of treatment, dieldrin was able to reduce NMDAR and type-I metabotropic GluR functionalities. The NMDAR immunolabelling revealed that it undergoes internalization after 6 DIV of exposure to dieldrin. Whereas mGLUR1 expression remained unaffected, mGLUR5 was down-regulated after the longer treatment. In contrast no changes were observed neither on receptor functionality nor on the receptor levels after 2 DIV of exposure to dieldrin. However double immunostaining for NMDA and mGLUR5 showed that these receptors loosed colocalization on the cell membrane in cells treated with dieldrin either for 2 and 6 DIV.

Conclusions: We confirmed that the permanent blockade of the $GABA_A$ receptor by this persistent pesticide triggers adaptative neuronal changes consisting on the reduction of the glutamatergic transmission. Moreover it seems that the presence of mGLUR5 plays crucial role on the glutamate excitotoxicity in cortical neurons. Altough these results could attribute protective effects to this pollutant in a excitotoxic model, it seems properly that, through interfering with the glutamatergic transmission, dieldrin might impair memory and learning and could also alter behaviour.

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(DEVELOPMENTAL) NEUROTOXICITY – A TEST CASE FOR THE TOXICOLOGY IN THE 21ST CENTURY?

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The US National Academy of Science has put forward two years ago a vision for toxicity testing in the 21st century, which should be based primarily on human cell systems and pathways of toxicity testing. US EPA has adopted this vision in their toxicity testing strategy from March 2009. The challenge ahead is now, to device this new type of safety assessments, assess its utility for regulatory purposes and implement change.

The presentation will highlight the background of this vision, the experiences in the field of alternative methods, which have contributed to this paradigm-shift, and lay out the steps ahead. The test case of (developmental) neurotoxicity will be used to characterize the challenges and to take stock of achievements so far.

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GENE EXPRESSION AS A MARKER TO DETECT DEVELOPMENTAL NEUROTOXICITY USING PRIMARY NEURONAL CULTURE OF RAT CEREBELLAR GRANULE CELLS

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Purpose. The major advantage of primary neuronal cultures for developmental neurotoxicity testing (DNT) is their ability to replicate the crucial stages of neurodevelopment. In our studies using primary culture of cerebellar granule cells (CGCs) we have evaluated whether the gene expression relevant to the most critical developmental processes such as neuronal differentiation (NF-68 and NF-200) and functional maturation (NMDA and $GABA_A$ receptor), proliferation and differentiation of astrocytes (GFAP and S100β) as well as presence of neural precursor cells (nestin and Sox10) could be used as a endpoint for in vitro DNT.

Methods. The expression of these genes was assessed after the exposure to various pesticides (parathion, dichlorvos, parathion, dichlorvos, pentachlorophenol and cycloheximide) that could induce developmental neurotoxicity through different mechanisms.

Results. All studied pesticides significantly modified the expression of selected genes, related to neuronal and/or glial different stages of cell development and maturation. The most significant changes were observed after the exposure to paraquate and parathion (down regulation of mRNA expression of NF-68 and NF-200, NMDA and GABAA). Similarly dichlorvos affected mainly neurons (decreased mRNA expression of NF-68 and GABAA receptors) whereas cycloheximide had an effect on both neurons and astrocytes as significant decrease in the mRNA expression of both neurofilaments (NF-68 and NF-200) and astrocyte markęrS100beta) was observed.

Conclusion. Our results suggest that toxicity induced by pesticides that target multiple pathways of neurodevelopment can be identified by studying genes expression that are involved in different stages of cell development and maturation and could be used as a sensitive endpoint for initial screening to identify the compounds with potency to cause developmental neurotoxicity.

HUMAN NEURAL STEM CELL LINE AS AN ALTERNATIVE MODEL FOR IN VITRO DEVELOPMENTAL NEUROTOXICITY TESTING

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Purpose: Our aim was to investigate whether a human neural stem cell line, derived from umbilical cord blood, can serve as a reliable test model for DNT,

Methods: Cells of the **h**uman **u**mbilical **c**ord **b**lood-derived **n**eural **s**tem **c**ell line (HUCB-NSC) were cultured and tested at different developmental stages, ranging from non-differentiated to committed neural progenitors, and differentiating neuronal, astroglial and oligodendroglial cells. Conventional methods and emerging nano/micro technologies were used for toxicological screening. The selected compounds - neurotoxic (sodium tellurite, methylmercury chloride, cadmium chloride, chlorpyrifos and L-glutamate) and non-neurotoxic (acetaminophen, theophylline and D-glutamate) were applied at different concentrations. Cytotoxicity, proliferation, apoptosis and lineage differentiation were the main endpoints analyzed.

Results: HUCB-NSCs were differentiated into neural lineages by defined exposure to selected growth factors and neuromorphogenes. Dose range finding for tested compounds allowed windows of exposure at non-cytotoxic concentrations to be established. Less differentiated HUCB-NSCs were generally more sensitive to neurotoxicants with the notable exception of L-Glu, which showed higher toxicity to later stages. The relative potency of the compounds was CdCl2>MeHgCl>>CPF>>L-Glu. A low dose of MeHgCl (0·05□M) inhibited cell proliferation and induced apoptosis significantly. At the differentiated stage 1 □M MeHgCl induced selective break down of astrocytic cells. 1mM L-Glu did not influence early stages of HUCB-NSC development, but did affect cells directed into neurons at their late stage of differentiation. Non-neurotoxic compounds did not affect HUCB-NSC key developmental processes. Experiments on biofunctionalized surfaces revealed the importance of stem cell/surface interactions and protein-type dependent response of HUCB-NSC to MeHgCl toxicity.

Conclusions: The HUCB-NSC cell line is highly sensitive to even low doses of neurotoxicants but generally not sensitive to non-neurotoxic compounds; it can detect adverse effects in a developmental stage- and cell type-dependent manner. Thus it can serve as a human stem cell-based *in vitro* model for DNT testing.

IN VITRO NEURAL TISSUE FOR REPLACEMENT OF TRANSGENIC ANIMALS WITH MEMORY/LEARNING DEFICIENCIES

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The goal of the project is to develop and evaluate an in vitro system composed of a network of synaptically connected neurons. The system could replace the memory/learning tests of drugs and neurotoxic compounds that are performed in animals. It could also be used as a memory/learning disease model system to replace studies performed in transgenic animals. The first phase of the project has been successfully completed. In the first phase we have developed and tested the components of the system and we have assembled them in an integral system. The work performed in the first phase has as follows: Neurons have been generated from embryonic stem cells using different differentiation protocols. Their properties have been checked with the expression of gene markers and global gene expression analysis. Hydrogels in which the neurons can attach and grow have been developed and their physical properties determined and optimized for the cell growth. Molecules that affect the cell attachment and dendrite development have been used in the hydrogels in order to control the neurons behaviour and synaptic network development. Perfusion bioreactor systems that carry the hydrogels seeded with neurons have been installed and their operation has been optimized to assure long term cell viability. Microelectrode arrays have been incorporated in the bioreactors in contact with the neurons of the hydrogel. The electrodes have been connected with a system for electrical stimulation, response signal amplification, noise extraction, spike detection and data storage, (MEA system). New algorithms for statistical analysis of the response signal have been developed and installed in the system. The system has been checked in experiments with neurons inside hydrogels and signals have been recorded and analysed.

In the second phase the system will be stimulated to acquire the memory of the stimulus and various tests will be performed. Neurons generated from new normal as well as transgenic embryonic stem cell lines that have been developed will be used in the system to compare the normal and transgenic in vitro neural tissue that will develop in the developed system. Experiments with neurotoxic compounds, selected during the first period of the project, that influence memory will be performed in the system and their effect on the ability of a normal synaptic network to memorize electrical stimuli will be determined. It is envisaged that further development of the in vitro neuronal tissue system will incorporate in this additional high level functions besides the memory/learning, bridging the gap between in vivo and in vitro.

ARE THE NEUROTOXIC EFFECT OF LOW-LEVEL LONG-TERM EXPOSURE TO ORGANOPHOSPHATES PERMANENT?

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Purpose: The Hula valley in the Upper Galilee has been extensively cultivated during the last five decades since its swamps were drained. Between 1987 and 1991, our studies which were supported by the World Health Organization assessed the low-level long-term exposures to OP pesticides and the consequent long-term neurological and neurobehavioral effects in workers and residents in several kibbutzim in the valley. We are currently continuing the work started on the original cohorts of 200 individuals more than 20 years ago to assess the extended outcome of prolonged everyday OP exposure. This is the first study with such a long follow-up.

Methods: We have generated a strong database of the original cohorts. Cognitive tests, neurophysiology and blood biochemistry studies including paroxonase-1 and neuropathy target esterase, and urinary OP metabolites measurements are under way.

Results: The original cohorts showed symptoms and signs of neurobehavioral dysfunction, with dose-response gradients between the symptoms and the measured levels of OP metabolites in urine samples of kibbutz residents, including children. Electrophysiological tests demonstrated mildly impaired nerve conduction velocities and amplitudes in both sensory and motor nerves. Neurobehavioral tests showed changes in measures of mood, and mental and motor performance. Measures of short-term memory, attention and time to reaction performance were impaired more in field workers than in other residents of the kibbutzim. Preliminary results of the current cognitive tests show mild signs of impairment in the performance of executive tasks of kibbutz residents.

Conclusions: These findings significantly contribute to the existing knowledge on the deleterious late cognitive effects of prolonged everyday long-term low-level exposure to environmental OP pesticides. The methodology of the study provides a template for introducing epidemiologic approaches to neurobehavioral toxicology in Israel. The final results will allow to guide planning and regulation of OP use in agriculture.

SUPPRESSION OF OXIDATIVE INJURY AND NEURODEGENERATION IN CHOLINERGIC TOXICITY

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Purpose: Prophylactic agents acutely administered in response to anticholinesterases intoxication can prevent toxic symptoms, including fasciculations, seizures, convulsions and death. However, anticholinesterases also have long-term unknown pathophysiological effects making rational prophylaxis/treatment problematic. We have used organophosphate DFP (diisopropylphosphorofluoridate) as a model for nerve agents and pesticide organophosphate acetylcholinesterase inhibition. Specifically, we tested the hypothesis that DFP-induced seizures, reactive oxygen and nitrogen species (ROS/RNS) generation and alterations in ATP mediate its neuronal injury.

Method: We have evaluated DFP-induced changes in biomarkers of oxidative damage, isoprostanes (F_2-IsoPs, and F_4-NeuroPs), nitric oxide (citrulline), high-energy phosphates (HEP; ATP, PCr), indicators of inflammatory response (PGE_2) and degeneration of the pyramidal neurons in the CA1 hippocampal area. We have also investigated whether treatment with antioxidants and memantine attenuate biomarkers of oxidative damage and associated dendritic degeneration of pyramidal neurons in the CA1 hippocampal area.

Results: Rats treated with DFP (1.25 mg/kg, s.c.) developed onset of toxicity signs within 10-15 min, which progressed to maximal severity of seizures and fasciculations within 60 min. At this time point, biomarkers of cerebral ROS generation, F_2-IsoPs and F_4-NeuroPs were significantly increased ($p<0.01$) to 142% and 225% of control, respectively. Severe seizures also induced three fold elevation of citrulline followed by significant ($p<0.001$) depletion in ATP in rat cerebrum. Quantitative morphometric analysis of pyramidal neurons of the hippocampal CA1 region 1 h following DFP exposure revealed significant decreases ($p<0.01$) in dendritic lengths and spine density to 30% and 58% of control, respectively. When rats were pretreated 30 min before DFP exposure with the antioxidants alpha-phenyl-N-tert-butylnitrone (PBN, 200 mg/kg, i.p.), Vitamine E (100 mg/kg, i.p./day for 3 days) or memantine (18 mg/kg, i.p.) a significant attenuation in DFP-induced increases in F_2-IsoPs, F_4-NeuroPs, citrulline and depletion of HEP was noted. Furthermore, attenuation of biomarkers of oxidative damage following antioxidant or memantine pretreatment was accompanied by rescue from dendritic degeneration of pyramidal neurons in the CA1 hippocampal area.

Conclusion: These findings support a prominent role of antioxidants and memantine in promoting neuronal survival following anticholinesterase-induced seizures (Supported by NINDS NS057223).

MIXED LINEAGE KINASE 3 SIGNALING: RELEVANCE TO NEUROINFLAMMATORY PROCESSES IN NEUROTOXIC CELL DEATH

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The Mixed Lineage Kinase (MLK) family members are unique in the sense that they contain signature sequences of both Serine/Threonine and Tyrosine Kinases in their catalytic domain. Although the MLK family members were initially identified as markers for specific types of cancer, recent studies show that they play a critical role in dopaminergic neuronal loss and thus could serve as targets for Parkinson's disease (PD). The mechanism by which MLKs cause cell death, however, is still not well understood. Our results suggest that MLK3, a MLK family member can activate Jun-N-terminal Kinase (JNK) under conditions of stress and promote cell death. MLK3 itself can be regulated by Glycogen Synthase Kinase3β (GSK3β) under stress condition. We also observed that MLK3 kinase activity was down regulated via PI3K-AKT pathway under normal (growth factor sufficient) conditions, suggesting the possibility that MLK3 activation during cellular stress could lead to neuronal loss. Furthermore, we also identified the pro-inflammatory cytokine TNFα and the pro-apoptotic lipid, ceramide as specific agonists of MLK3. These ligands are known to be elevated in PD patients, suggesting a potential activation of MLK3/MLKs by pro-inflammatory pathways during PD pathogenesis. Most of the data related to the role of MLKs in PD were derived utilizing the pan-MLK inhibitor, CEP-1347, that did not directly implicate a specific MLK isoform in the pathogenesis of PD. Our recent studies with MLK3 knockout mice showed a significant protection of dopaminergic neurons and motor function in response to MPTP intoxication. Collectively these studies show that targeting MLK3 signaling pathway may be therapeutically beneficial to halt dopaminergic neuronal loss in PD. Supported by (NIH grants: R01GM 055835, NS 38644, ES10586 and NS 0365167).

SNAKE VENOM ANGIONEURINS: TOXIC OR THERAPEUTIC GROWTH FACTORS?

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Angioneurins are molecules that affect both neural and vascular cell processes such as nerve growth factor (NGF), which induces neuronal differentiation and vascular endothelial growth factor (VEGF), which initiates the angiogenic process of blood capillaries. We have recently isolated and characterized both NGF and VEGF-like compounds from snake venoms using two steps of reverse phase HPLC. Both these factors are able to induce *in vivo* plasma extravasation by causing a general microvessel leakage and *in vitro* increased permeability (reduction in electrical resistance) of brain capillary endothelial monolayer. These properties strongly suggest that these growth factors have an offensive role in improving the pharmacokinetic properties of snake venom neurotoxins and toxic enzymes upon intoxication. In contrast to these destructive properties, snake venom NGF and VEGF potently stimulate endothelial cells proliferation in a TrkA and VEGFR-2 receptors dependent manner, respectively. This stimulatory effect on endothelial cells from different vascular beds was correlated with activation of MAPK signaling pathway. *In vivo*, these angioneurins induced angiogenesis in a Japanese quail assay and in a Matrigel plug assay in mice. Similar to snake venom and human recombinant VEGF, snake venom NGFs promoted migration, capillary sprouting and other angiogenic parameters. Recently, alpha9beta1 integrin, known to interact with VEGF isoforms, was also found as a receptor for NGF, providing a novel target and explanation for angioneurins-induced migration of endothelial cells. These properties render snake venom angioneurins promising lead compounds in therapeutic angiogenesis for certain cardiovascular diseases.

FROM MUSTARD GAS ANTIDOTE TO POTENTIAL ORAL TREATMENT OF NEUROINFLAMMATION

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Topical application of iodine formulation protects against chemical- and heat-induced skin lesions. It was proposed that substance P (SP), that plays a key role in neuronal transmission, inflammation and skin burn evolution, is oxidized by iodine to form its sulfoxide form, resulting in reduction in the inflammatory skin response. While determining levels of SP and its oxidation product in iodine-treated skin burn, we discovered a novel nonapeptide that showed counter-irritating effects against mustard gas- and heat-induced skin lesions. This antiinflammatory effect led us to test the efficacy of a peptide analog on neuroinflammatory animal systems. Oral administration of the peptide significantly reduced the neurological score in both the active and adoptive transfer models in mice. Structural studies revealed that slight changes in the peptide reduced its activity. CD3 ζ-chain expression, a T cell receptor component which plays a key role in receptor assembly, expression and signaling, was downregulated in cells derived from peptide-treated mice. The peptide-induced impaired immune function might be of therapeutic value in neurological inflammatory disorders such as MS and other autoimmune diseases like psoriasis, rheumatoid arthritis and systemic lupus erythematosus.

THE IMPACTS OF INDUSTRIAL POLLUTION

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Purpose: The Haifa and Akko (Acre) subdistricts of the Northernwestern area of Israel have been main sites of the country's heavy and chemical industries, initiated some eight decades ago. For most of this time period, there were little or no measures taken to reduce and control emissions of air pollutants from these facilities. The aim of this study was to estimate the historical release of major organic (1930's-2007) and inorganic air toxics, their dispersion and deposition in the Haifa Bay area, an area of about 300 km^2.

Methods: Analysis of metals emissions: the historical records of residual oil no.6 fuel combustion were collected, then, using available published data we estimated the emission of the metals emitted. A validation for these analyses was conducted by sampling and analyzing metals in the soils of Haifa Bay area. Soil samples collected as part of a geochemical study of the area were analyzed with ICP-AES, according to natural and synthetic international standards. The data collected was presented in ArcInfo 9.3.In addition, in 2007 air sampling of particulate matter less than 10 μm and less than 2.5 μm (PM_{10}, and $PM_{2.5}$) was undertaken, and their metal content analyzed. Analysis of organic emissions: emissions from an iron and steel recycling facilities were considered as were those from polyvinyl-chloride (PVC) factory. Also estimations for VOC's emission from Haifa refineries were considered.

Results: Analysis of all the above data suggests that metals are being carried primarily with the $PM_{2.5}$ fraction, and can be dispersed in large quantities for 10-15 kilometers from their source. These metals include known neurotoxins such as lead, manganese and mercury. Nickel and vanadium were also dispersed in large quantities. Other metals released were transition elements such as copper, iron and zinc.
Regarding organic emissions, compounds such as Dioxins and Furans, PCB's, PAH's, ethylene dichloride, and vinyl chloride monomer were released and dispersed at extremely high rates.

Conclusions: This study confirms that the residents in Haifa Bay area were subjected to massive air pollution and for over half a century. All the major air toxics were released at extremely large quantities, in some cases reaching 4 orders of magnitude greater than what is attainable. Pollutants included neurotoxins, organic and inorganic carcinogens as well as transition metals. To better understand the effects on the exposed population, a further study of neurotoxicity and morbidity is required.

EXPOSURE AND SUSCEPTIBILITY: SCHIZOPHRENIA IN A YOUNG MAN FOLLOWING PROLONGED HIGH EXPOSURES TO ORGANIC SOLVENTS

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A 25 year old man, the 9th of 10 sibs, whose family came to Israel from Tajikistan, was hospitalized for an attack of severe psychosis, requiring several months of hospitalization. He presented with a several month history of severe headaches, dizziness, nausea, headache and restlessness, delirium following several months of work in a paint factory, where he was exposed to many organic solvents for up to 17 h/d over a several month period at extremely high levels under very poor working conditions. His discharge diagnosis was psychosis, either from toxic or functional cause. Following several relapses and repeated hospitalizations, he was diagnosed several months later as suffering from paranoiac schizophrenia He is unable to work, spends much time in bed, suffers from concentration problems, cannot concentrates on reading, and was described as lacking motivation, suffering from chronic fatigue, and depressed. On PE, he appeared apathetic, there were no disturbances in organization of thoughts, concepts, judgment and perception, and there was no disorientation in relation to time, place or self. EEG's and brain scans were all unremarkable, but transaminase levels were elevated on first hospitalizations.

He requires sedatives, antidepressants, tranquilizers. A twin sister died after birth in Tajikistan. A 20 year old brother, one year after immigrating to Israel, was found dead after disappearing, without a known prior psychiatric history.

The fact that the patient's acute symptoms appeared following his sustained and massive exposure to organic solvents suggested the hypothesis that these exposures triggered the expression of underlying pre-existing susceptibility to schizophrenia, possibly on a familial basis. This hypothesis was the basis of a court-based settlement recognizing the cause-effect relationship between his exposures and the organic brain syndrome, the signs and symptoms of which were the first stage of his schizophrenia. The patient's case history states the case for careful occupational histories of past exposures in patients with schizophrenia. Case control studies are needed to explore the contributory role of neurotoxic exposures in triggering expression of the condition in susceptible individuals. We ask: Do current models of pathogenesis of this condition adequately reflect the possible role of such exposures?

ROLE OF PESTICIDE EXPOSURE IN PANDEMIC OF DEVELOPMENTAL NEUROTOXICITY

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The developing brain is particularly vulnerable to neurotoxicity. Although the sensitivity of the brain is well documented, the effect of individual pollutants is poorly documented. In a recent review (Lancet 2006; 368: 2167-78), we identified over 200 industrial chemicals known to cause brain toxicity in humans. All of them are also suspected to harm the developing brain, and about half of these chemicals are produced in high volumes. However, only a small number of industrial chemicals – lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene – are recognised causes of developmental brain dysfunction. These substances contribute to a pandemic of developmental neurotoxicity that appears to be 'silent', i.e., by escaping detection. New evidence is emerging in regard to developmental neurotoxicity caused by pesticides, which may add to this silent pandemic. We chose to carry out cross-sectional studies in a floriculture area of northern Ecuador, where the main occupation among women is in greenhouses. In two studies, we interviewed mothers of children in first and second grade about their occupation and pesticide exposure during pregnancy. The children underwent physical examination and were examined by a range of neurobehavioral tests. Current pesticide exposure was evaluated from cholinesterase activity and urinary pesticide metabolites. In both studies, children with maternal exposure to pesticides during pregnancy were at higher risk than unexposed children to show deficits in neuropsychological functioning, especially in motor speed and visuospatial functioning. In addition, exposed children showed higher blood pressure. The children's current pesticide exposure and paternal exposure showed less clear, if any, associations with the neurobehavioral outcomes. These findings support the notion that many more chemicals than the five listed above may be associated with developmental neurotoxicity. Systematic testing for neurotoxicity is lacking, but more than one thousand chemicals have been reported to be neurotoxic in laboratory models. New, precautionary approaches are therefore required for chemical testing and control that recognise the unique vulnerability of the developing brain.

EFFECTS ON COGNITIVE ABILITY FROM CHRONIC EXPOSURE TO SOLVENT BASED-PAINT

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Purpose: Construction painters use solvent based paint because its properties better protect metal structures in the outside environment. However, use of these paints results in chronic exposure to solvent mixtures that are neurotoxic.

Methods: Possible adverse cognitive effects were evaluated in a cohort of solvent exposed painters (94) and matched controls (74). Each participant completed: 1) physical examination, 2) questionnaires on exposure history and lifetime use of alcohol and drugs; 3) neurobehavioral performance measures. The exposures assigned were based on Monte Carlo techniques to estimate individual lifetime exposure using: current field air measurement and literature values during different painting applications; detailed exposure history questionnaire, historical paint composition, and protective equipment efficiencies. The neurological decrements were assessed using Cambridge Neuropsychological Test Automated Battery (CANTAB) and Neurobehavioral Evaluation System screening tests. The presence or absence of polymorphisms in genes controlling the metabolism of solvents were determined to evaluate whether metabolic capabilities altered an individual's susceptibility by genotyping 28 SNPs in genes associated with 11 metabolic enzymes of solvent neurotoxicants.

Results: Exposed and controls were high school educated, predominantly Caucasian (80%) males with an average age of 44 years, a minimum of 10 years working in their profession, and comparable lifetime alcohol use. The solvent-exposed subjects performed worse on tests of motor and visuomotor speed (finger tap, 5 choice reaction time), attention (match to sample visual search), verbal and visual learning and memory (paired associate, spatial recognition), and problem solving (Stocking of Cambridge) ($p < .05$ all tests). Preliminary analysis of the dose-response relationship between chronic exposure to paint solvents and neurobehavioral impairment among painters was found to be modified by the gene-environment interaction for two polymorphic genes CYP1A2 and NAT2.

Conclusion: chronic exposure to solvents from painting affects decreased attention span and some aspects of memory and the extent of the effect showed a gene-environmental interaction.

GENES, ENVIRONMENT AND SUSCEPTIBILITY IN PARKINSON'S DISEASE

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The earliest experts proclaimed Parkinson disease (PD) to be an environmental disease, including James Parkinson himself. But over the last 10 years we have seen genetics leap to the forefront of PD causality, with much research centered on genetic contributions. But in reality the crossroads between the two is likely one of the most traveled roads to the disease, as toxicity may be a relative term that is determined by each individual's genome. That is, the effects of environmental toxins and agents are often highly modulated by the form (allele) of an interacting gene in the individuals genome. While many of these are predictable, such as the P450 genes, the majority are not, and are best identified through modern genomic techniques. Several such examples exist, such as those involving the nitric oxide genes NOS1 and NOS2. An overview and examples of these interactions will be presented.

TOXICANT-INDUCED ALPHA-SYNUCLEIN MODIFICATIONS AS A MODEL OF GENE-ENVIRONMENT INTERACTIONS IN PARKINSON'S DISEASE

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Both clinical and experimental evidence consistently points to alpha-synuclein as a key player in the pathogenesis of Parkinson's disease (PD) and other neurodegenerative disorders (collectively referred to as "synucleinopathies"), justifying the view that this protein represents a promising new target for therapeutic intervention in these diseases. The precise mechanisms by which an endogenously expressed protein like alpha-synuclein becomes involved in pathologic processes are still not completely understood. An intriguing clue can be drawn, however, from human genetic studies showing that enhanced alpha-synuclein expression due to multiplication mutations of the alpha-synuclein gene is associated with familial parkinsonism and dementia. This observation suggests that any condition (besides genomic multiplications) capable of augmenting alpha-synuclein levels may contribute to the pathogenesis of PD and other synucleinopathies. Data will be presented showing that toxic agents damaging the nigrostriatal system (this system is particularly vulnerable to the neurodegenerative process of PD) cause an up-regulation of alpha-synuclein and modifications of the protein (e.g., nitration and aggregation) similar to those observed in post-mortem PD brains. These data support the hypothesis that toxic insults could contribute to the pathogenesis of synucleinopathies by increasing alpha-synuclein level within neurons. We will also discuss two potential strategies against the pathological accumulation of alpha-synuclein, one using RNA interference (RNAi) as a means to lower alpha-synuclein expression and the other involving lysosomal pathways of protein degradation that, once induced, could facilitate alpha-synuclein clearance. RNAi targeting alpha-synuclein and drugs acting as inducers of protein degradation could ultimately become valuable strategies for neuroprotective intervention in PD and other human synucleinopathies.

SELECTIVE ALTERATIONS IN THE GENE EXPRESSION, TRANSCRIPTION FACTORS AND MITOCHONDRIAL MEMBRANE PROTEOME IN MPTP-INDUCED MODEL OF PARKINSON'S DISEASE

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Parkinson disease (PD) is a common neurodegenerative disease characterizes by the progressive loss of midbrain dopaminergic neurons with unknown etiology. MPP^+ (1-methyl-4-phenylpyridinenium) is an active metabolite of neurotoxin 1-methyl-4-pheny-1,2,3,6-tetrahydropyridine (MPTP). The mechanism of toxicity is thought to involve the accumulation of the MPP^+ into mitochondria where it inhibits complex I of the respiratory chain resulting in impaired energy production and increased free radical production. In this study, we utilized variety of cell lines along with mice model of PD. PC-12 or a dopaminergic cell line MN9D cells were treated with MPP^+ or adult mice (C57BL/6N) were treated with 4 X 10mg/kg, ip, doses of MPTP two hours apart, 24 hours before sacrifice. MPP^+ produced a significant depletion of dopamine in PC 12 and MN9D cells line. A similar decreased of striatal dopamine was observed after MPTP treatment. Real time RT-PCR was used to examine selective genes of dopaminergic system in PC-12, MN9D cells, as well as in mouse substantial nigra (SN). MPP^+ and MPTP produced a selective alterations in different genes associated with dopaminergic system such as tyrosine hydroxylase, dopamine transporter and vesicular monoamine transporter. Significant changes were also observed in α -synuclein in both PC 12 cells and in SN of mouse after treatment with MPTP. In addition, we found that MPP^+ also significantly alters other novel genes associated with oxidative stress and apoptosis in MN9D cells. Furthermore, a novel method was developed to isolate mitochondrial membrane proteins for analysis by mass spectrometry. Highly pure mitochondrial fractions were isolated from MN9D cells or mouse brains following homogenization and centrifugation through Percoll™ gradients. Mitochondrial membranes were isolated by a sodium carbonate procedure before further purification and analysis. The labeled peptides were release from solid-phase supported by UV photocleavage and analyzed by liquid chromatography-landem mass spectrometry (LC/MS/MS). Utilizing the improved PhIST approach, 63 phospoproteins were identified by peptides derivatives with either light ($^{12}C_6$, ^{14}N) or heavy ($^{12}C_6$, ^{14}N) PhIST tags. The detected proteins belong to several functional classes that include zinc-finger proteins, receptor and transporter proteins. Apoptosis and ATP-related proteins. This analysis revealed significant alterations to the mitochondrial membrane protein profile following treatment with MPP^+ or MPTP. These finding suggest that MPP^+ and MPTP-induced neurotoxicity in *in vitro* as well as *in vivo* could be used a potential model to further explore the roles of these genes, transcription factors and proteins in the pathogenesis and potential treatment of PD.

ROLE OF A UBIQUITOUS NEUROTOXIC NON-PROTEIN AMINO ACID, BMAA, AND CYANOBACTERIA AS AN ENVIRONMENTAL CAUSE OF ALS, ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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Paul Cox and colleagues have demonstrated that cyanobacteria in the roots of the cycad palm produce a neurotoxic non-protein amino acid, BMAA, that is bio-concentrated up the food chain to humans suffering from the Guamanian type of 'neurodegenerative' disease, the ALS/Parkinson-Dementia Complex. Also, they have shown that : (1) Cyanobacteria producing neurotoxins, including BMAA, are concentrated in the estuary and source of drinking water of Hohara village in the Kii Peninsula of Japan, where the incidence of ALS/PDC is at least as high as in Guam. (2) Cyanobacteria producing BMAA are ubiquitous throughout the world. (3) Cyanobacteria are associated with 'algal blooms' in areas of water associated with human use and animal deaths.

Deborah Mash and colleagues in the University of Miami have independently confirmed that protein-bound BMAA is present in the brains of Florida, USA patients with Alzheimer's disease (12 cases, mean level of 111 ± 14.6 µg/g), with Parkinson's disease (2 cases, 176 and 218 µg/g), and with amyotrophic lateral sclerosis (13 brains, mean level of 134 ± 12.8 µg/g; 4 spinal cords, mean level 124 ± 69 µg/g). Age-matched brains of patients dying with non-neurological conditions contained only trace amounts of BMAA (12 cases with two specimens, 22 negative and 2 positive – 45 and 36 µg/g). Brains from Huntington disease patients, used as disease controls, contained only trace amounts of BMAA (8 cases, 7 negative and one positive – 11 µg/g).

Our continuing studies are investigating the roles of environmental exposure and genetic predisposition in producing this disease-related accumulation of BMAA, the mechanism of individual accumulation of BMAA in brain proteins, and the production of animal models of BMAA neuro-intoxication. Therapeutic opportunities based upon the BMAA hypothesis of the environmental neurotoxin responsible for age-related neurodegenerations will be discussed.

SODIUM TITANATE NANOWIRES: FABRICATION AND INTERACTION WITH PC-12 CELLS

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Recently there has been a surge in the development and use of nanomaterials in medicine. In the present study, sodium titanate NWs were created on the surface of titanium (Ti) foil, because this titanate belongs to the family of TiO_2 that has been extensively studied for making many different biomedical devices such as hip replacements and stents. The Ti foil was incubated with sodium hydroxide at different temperatures (150ºC and 240ºC) for 2, and 6 hours. After the reaction, the NWs were gold plated for morphology characterization under a scanning electron microscope (SEM). Incubation of the Ti foil at various times and temperatures produced different NWs, with longer incubation times combined with higher temperature creating longer nanofibers. These NWs were then exposed to PC-12 cells, a rat pheochromocytoma cell line that secretes catecholamines and has been used to evaluate dopaminergic neurotoxicity, for 24, 48 and 72 hours. After the incubation, the growth pattern and morphology of PC-12 cells grown on these NWs were fixed with paraformaldehyde and gold plated, and then evaluated under the SEM. The remainder of the cells were processed to measure the dopamine content using HPLC/EC. Compared to others, the NWs formed at 240°C for 6 hours would promote the growth of densely populated PC-12 cells. Incubation of these cells with the NWs did not appear to produce cytotoxicity as measured by the concentration of dopamine at 24, 48 and 72 hours. In conclusion, these data suggest that by manipulating reaction conditions, the fabrication of the NWs can be controlled and these NWs do not produce toxicity in a cell line commonly used to evaluate alterations in the dopaminergic system. This finding may have an application in the field of nanomedicine and in particular neuroscience.

DEVELOPMENTAL HYPOTHYROIDISM ALTERS VISUAL FUNCTION OF ADULT RATS

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Purpose. Many environmental agents alter thyroid hormone status, which is essential for neural development. The dose-response relationships for such effects are unclear, however, because most of the existing literature involves complete thyroid ablation, whereas environmental exposures typically cause only modest thyroid disruption. This study was intended to evaluate dose-response relationships of graded levels of thyroid hormone insufficiency during development on the function of the mature visual system.

Methods. Pregnant Long-Evans rats were given 0, 1, 2, or 3 ppm of the thyroid hormone synthesis inhibitor propylthiouracil (PTU) in their drinking water from gestation (GD6) through lactation (PND21). This dose range produces graded reductions in circulating levels of T4 20-50% in dams and pups. At ~60 days of age, electrodes were surgically implanted over visual cortex into 1 or 2 male pups/litter. One week later the visual evoked potentials (VEPs) were recorded from each rat using modulating visual patterns (0.16 cpd) at 0, 8, 12, 16, 24, 32, 48, 64, and 80% contrast. The VEP amplitude at twice the stimulus rate (F2), expressed as a function of log stimulus contrast, was used to generate contrast-gain functions, for which the zero-amplitude intercept is interpretable as visual contrast threshold.

Results. The VEP F2 amplitude was significantly reduced in rats treated with PTU. The slope of contrast-amplitude functions was progressively reduced at each successively higher dose.

Conclusions. The results suggest that modest reductions in thyroid function limited to the perinatal period are sufficient to permanently alter visual system function in adulthood. These findings are consistent with visual contrast sensitivity deficits seen in children with congenital hypothyroidism. Notably, deficits in hippocampal synaptic function were observed at similar dose levels, suggesting that modest developmental hypothyroidism leads to global physiological deficits in the CNS.

This abstract does not reflect EPA policy.

DEVELOPING AN EXPOSURE-DOSE-RESPONSE MODEL FOR ORGANIC SOLVENTS: PROGRESS ON MODELING *IN VIVO* DOSE-EFFECT RELATIONSHIPS

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We are developing an exposure-dose-response model for volatile organic compounds (VOCs) to predict acute effects of VOCs on nervous system function from exposure data (concentration and duration of inhalation).

Pharmacokinetic modeling enables accurate estimates of internal doses in the brain, and dosimetric studies have confirmed that the concentration of the solvent in the brain predicts the acute effects. Studies *in vitro* show that VOCs reliably perturb flux through neuronal ion channels, but the dynamic links between these effects on ion channels and the neurobehavioral and neurophysiological effects of those perturbations *in vivo* are not understood. Neural network modeling of visual signal detection, a behavior reliably impaired by VOCs, has identified a role for classically-conditioned associations in this behavior, and may help identify the neural pathways mediating effects of VOCs on the behavior. Despite the limited pharmacodynamic knowledge of how effects on ion channels translate into functional changes, analysis of dose-effect functions *in vivo* enable useful quantitative comparisons of efficacy and potency across VOCs, endpoints, and species. A meta-analysis of acute effects of inhaled VOCs *in vivo* revealed that dose-effect functions were statistically indistinguishable for toluene, trichloroethylene, perchloroethylene, and 1,1,1-trichloroethane, but differed significantly across endpoints (choice reaction time in humans and visual evoked potentials, visual signal detection, and shock avoidance in rats). This analysis revealed that the sensitivity of the endpoints mapped systematically across inferred levels of motivation engaged by the assessment methods, with rats and humans displaying equivalent sensitivity under conditions of low motivation. Nevertheless, some anomalies indicate the need for improved knowledge of the pathways linking effects on ion channels to those on function, and may in fact yield information critical for characterizing those pathways accurately. The abstract does not reflect EPA policy.

CHOLINERGIC AND GLUTAMERGIC RELATION IN ORGANO-PHOSPOPHATE (OP) POISONING. THERAPEUTIC CONSIDERATIONS

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Background and purpose. There is general agreement that OPs used in agriculture or warfare act by inactivation of the AchChE and induction of a pernicious cholinergic syndrome. This opinion in not contestable in spite some clinical discrepancies, in a conception in which the function of the Nerve System is managed exclusively by Adrenaline-Ach couple of neurotransmitters (NT). However, the recognition of Glutamate as having a high representation as NT in the brain (more than 70%), its main role in the excitotoxic syndrome and especially its involvement in the pathology of Beta Amyloid may change basically this approach. The experimentation **in vivo** with Amyloid components has become possible only for a few years, after publication of a study about the raising of Anti Amyloid Antibodies (AAA) and availability of these agents for common use (1) The relation between inactivation of ChE by OP on one hand and the AAA on the other hand is not yet fully clarified, however the binding of esteratic sites of muscles by AAA is an immuable evidence that does not belong to cholinergic pathology. In this light the revision of seemingly intangible dogmas is a justified challenge.

Methods. The affinity of AAA to Neuro Muscular Junctions mentioned is supposed to be paralleled by affinity for pathological Amyloid of blood or urine of Neurodegenerative Diseases. Pathologic samples from human Degenerative Diseases was collected in parallel from rats and mice submitted to parathion experiments. As in the Crystallography technique the samples had been examined by 'drop drying' method with certain modifications: the slide was maintained right for hours, temperature (37,6 C) controlled and protected of light because their charge of FIT charged antibodies. The samples have been mixed with antibodies, according the scheme of the experiment. After desiccation (generally after 4-6 hours) the samples had been examined by fluorescent microscope in case of mixing of the samples with FIT antibodies, or microscope with polarized light in case of staining the preparation with Congo Red.

Results; The results are representatives for samples of blood and urine of patients of AD, ALS and Schizophrenia (!) and in parallel mice and rats poisoned with an available laboratory OP in this case Parathion. The examination of the samples took into account the individual affinities of a panel of AAA, prepared differently and the direct examination of the Amyloid content (after CR staining). The elements taken into consideration have been the morphology of the image, color of the figurate elements, luminescence and others. Each image exposed in the Poster is accompanied by a detailed description .

Conclusion:

1. The findings are similar in the chronic human diseases or the poisoning with Parathion and prove in both cases the presence of Beta Amyloid, spontaneously or in deposition.
2. The effect is much enhanced under the effect AAA.

3. The deposition of Amyloid is carried out as a mass of amorphous material or big, complex crystals intermingled with non-amyloid materials, most probably the toxic agent.
4. Removing the toxic complex by extra corporeal dyalisis seems to be technical possible and offers theoretically a rational therapeutic for both pathologic issues.

1 S Calmanovici. Clinical expressions of Alzheimer's Disease. Israel Society for Histochemistry, Annual Meeting June 2007

BEHAVIORAL CHANGES AND β-AMYLOID LEVELS IN A MICE MODEL OF ALZHEIMER DISEASE AFTER AN ACUTE EXPOSURE TO CHLORPYRIFOS

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Purpose: Organophosphates (OP) exposure has been related to long term nervous system effects, in both humans and animals. Chlorpyrifos (CPF) is a cholinesterase (ChE) inhibitor OP insecticide, used in agricultural activities and household. In the present study, we evaluated the long term effects of an acute single exposure to CPF on brain β-amyloid levels and its relationship to cognitive impairment in transgenic male adult mice (Tg2576) carrying the Swedish mutation for familiar Alzheimer disease (AD).

Methods: At 7 months of age Tg2576 and their respective Wild type male mice were exposed s.c. to 0, or 50 mg/kg of CPF. Body weight changes were recorded during the experiment and ChE activity was measured in brain 72 hr after CPF treatment. Effects on spatial learning and memory were evaluated 17 and 30 weeks after the treatment in a Morris water maze test (MWM). Motor coordination and balance were tested using a rotarod 19 weeks after CPF administration. Brain cortical and hippocampal β-amyloid (Aβ40, Aβ42) levels were measured 8 months after CPF treatment, by ELISA.

Results: Results showed a body weight decrease and ChE inhibition percent of 52,27% 72 hours after the treatment. Differences were not observed between Tg2576 and wild-type during the acquisition of the spatial task; however treated transgenic mice showed a better retention of the task 17 weeks after the treatment but not 30 weeks after. The levels of β-amyloid were significantly increased in brain cortex and hippocampus of Tg mice 8 months after the administration of an acute dose of CPF.

Conclusions: A single dose of CPF exposure, producing a moderate ChE inhibition, improves transiently the retention of the spatial MWM task and increases both beta1-40 and 1-42 levels in cortex and hippocampus in treated Tg2576 mice.

SEARCH FOR BIOMARKERS TO CONTROL AND PREVENT THE RISK OF NEUROTOXICITY INDUCED IN RATS SUB-ACUTELY EXPOSED TO $MnCl_2$

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Purpose: Manganese (Mn) is an essential nutrient required for normal lipid, protein and carbohydrate metabolism. However excessive exposure to this metal may occur in the metallurgical, dry-cell battery, and mining industries, resulting in toxicity referred to as manganism. Manganism, a Parkinson's disease-like syndrome, is characterized by an extrapyramidal dysfunction with neuropsychiatric and motor symptoms. Chronic exposure to this metal is associated with elevated brain Mn accumulation especially the globus pallidus and striatum. Exposure assessment relies predominantly on the determination of Mn in blood and urine, both of which show poor relationships to exposure. Accordingly, a critical need exists for the development of better biomarkers as surrogates of Mn exposure. The aim of this study was to examine the relationship between Mn exposure and effect biomarkers, with emphasis on peripheral biomarkers of exposure.

Methods: Male Wistar rats (180-200 g) were exposed to several doses of Mn 10.0 mg/Kg (4 and 8 doses) via intraperitoneal injection. The exposure to Mn was evaluated by measuring Mn levels in both the brain and blood along with several biochemical end-points (see below).

Results: Mn levels in the brain were increased and significantly different from control ($p<0.001$) for both doses. Blood levels failed to reflect the dose- dependent increase in brain Mn, with only the 8 doses group showing significant differences ($p<0.0001$) compared to controls. GSH levels in the brain were significantly decreased in the 8 dose-treated animals ($p<0.0001$). Prolactin levels in serum were also analyzed as a surrogate measure of dopaminergic system integrity. A significant and dose-dependent increase in prolactin levels was found for both treatment groups ($p<0.0001$) compared to controls. In addition, a decrease in motor activity was observed in the 8-dose group.

Conclusions: Taken together, the present study demonstrates that (1) peripheral blood levels were poor indicators of Mn brain accumulation, (2) Mn reduces levels of brain GSH likely reflecting oxidative stress, and (3) Mn increases blood prolactin levels. The possibility that peripheral prolactin levels can serve as biomarkers of Mn exposure necessitates additional studies.

ENVIRONMENTAL NEUROTOXICOLOGY: A PRELIMINARY STUDY TOWARDS THE USE OF PETS AS SENTINELS OF HUMAN EXPOSURE TO NEUROTOXIC POLLUTANTS

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Purpose: Some papers are dealing with the use of dogs as sentinels of lead exposure for humans (Monkiewick 1998) and specially in order to prevent children saturnism (Ostrowsky 1990). As no such study has been performed in France, although children are still at risk for saturnism, we aimed to develop a parallel survey of dogs and children at the national French level for saturnism and other neurological diseases linked to exposure to environmental pollutants.

Methods: A preliminary approach was therefore necessary. In a sample of healthy dogs coming to the veterinary consultations, we have collected informations concerning the physiological parameters, the baseline value of blood lead concentration and the baseline blood level of organic pesticides known as neurotoxic hazards such as insecticides in the same dogs.
This study has been performed on the basis of 174 environmental questionnaires administered to dogs owners consulting in Alfort veterinary School. 161 dogs have successfully been sampled for toxicological analysis, and 160 for the biochemical and hematological analysis. We only were faced to 20 dog owners' refusals.

Results: The blood numeration and formula and biochemical parameters values were recorded. The biochemical parameters evaluate the renal, hepatic, pancreatic and muscular function. Also the thyroxin blood concentration was measured as a control of the thyroid function. Most of the results were included in IDEXX laboratory historical values. The mean blood lead level was 21.32+/- 20.41 µg/L. 109 dosages reached higher levels than the limit of quantification of the method (0.5 µg/L) but lower than 300µg/L.
For the neurotoxic insecticides, most of the samples (125 or 126) gave results under the limits of quantification of the chromatographic selected method : < 0, 01µg/g for organochlorine ; < 0, 025µg/g for carbamates and organophosphates ;< 0, 025µg/g for pyrethroïdes.Some few dogs living in a particular environment showed higher values that raised causality questions.

Conclusions/Perspectives: At the moment we haven't succeed in getting involved in a comparative epidemiological study for saturnism (canine and human). However, in further experiments we will be able to compare blood levels of such environmental pollutants in any other dogs to the ones from this first data base in order to stress any risk for humans of the same family, sharing the same environment. We also will be able to evaluate whether such contaminants could influence the physiological (biochemical or haematological) parameters of dogs, which have not been proved so far.

Eventually, at longer term we intend to motivate the French vets to get involved in a vet network created at the national level and working as an alarm system for human risk of environmental origin for different chronic diseases: neurological diseases but also CNS cancers.

TRANSGENIC EXPRESSION OF HUMAN TRUNCATED TAU PROTEIN INDUCES NEUROTOXICITY IN ANIMAL AND CELLULAR MODEL OF HUMAN TAUOPATHIES

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Purpose. Modifications of protein tau have been implicated in the pathogenesis Alzheimer's disease and related tauopathies. It has been accepted that various forms of tau protein, can lead to neurotoxicity. However detailed mechanisms of tau induced neurotoxicity are not completely understood. We have tested the hypothesis whether or not tau protein modification, specifically truncation could be an early step in human tauopathies and cause neurotoxicity in central nervous system.

Methods and Results. We have generated transgenic rats expressing human truncated tau protein and investigated the effect of tau truncation in neurons of rat brain and also in primary corticohippocampal neuron cultures. Using electron paramagnetic resonance we observed significantly increased accumulation of ascorbyl free radicals in brains of transgenic animals (up to 1.5-fold increase; $P<0.01$). Examination of an in vitro model of cultured rat corticohippocampal neurons revealed, that even relatively low expression of human truncated tau protein (equal to 50% of endogenous tau) induced oxidative stress that resulted in increased depolarization of mitochondria (~1.2-fold above control, $P<0.01$) and increases in reactive oxygen species (~1.3-fold above control, $P<0.001$). This is an underlying cause of increased susceptibility of truncated tau expressing neurons to externally applied oxidative stress. Furthermore we found that mitochondrial damage-associated oxidative stress may be an early event in neurodegeneration and using of common antioxidants we have significantly reduced a tau-induced elevation of ROS.

Conclusion. Our data indicate that truncation of tau may precede oxidative stress in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease and other tauopathies. These findings may have implications for the strategies aiming at prevention of neurofibrillary degeneration and cognitive decline, and identification of potential new targets for prevention of neurotoxicity in CNS.

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REVISED CELLULAR WORKING MECHANISM OF AMPHETAMINE

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Purpose: Amphetamine (AMPH) exposure increases dopamine (DA) brain levels, which is mainly due to reversal of the dopamine transporter (DAT) [1]. Few studies showed that AMPH exposure reduces the amount of DA released per vesicle [2] and increases the release frequency [3]. Although these two studies are somewhat contradictory, the results were combined resulting in the widely-accepted theory that AMPH causes leakage of DA and also Ca^{2+} from intracellular vesicles. The aim of our study was to verify this theory and identify possible other cellular effects of AMPH.

Methods: Exocytosis was recorded using amperometry [4] and changes in the intracellular calcium concentration $[Ca^{2+}]_i$ were determined using the calcium sensitive fluorescent ratio dye Fura-2 in PC12 and chromaffin cells. Cells were exposed to 10 or 100 µM D- or DL-AMPH for 15 minutes. For specific experiments cells were exposed to 100 µM L-dopa.

Results: Unexpectedly, 15 minute exposure to 10 or 100 µM D- or DL-AMPH does not decrease the content of dopamine per vesicle and does not evoke exocytosis in PC12 cells or chromaffin cells. Comparable results were obtained following exposure to AMPH for 45 minutes or at 37 °C. Additional measurements of $[Ca^{2+}]_i$ revealed that AMPH did not induce noticeable release of vesicular Ca^{2+}.
Vesicle content is increased up to ~140% in cells exposed to L-dopa prior to saline exposure. However, when these pre-treated cells are exposed to AMPH vesicle content drops to ~90%, indicating that under these specific conditions AMPH can cause vesicle leakage.

Conclusions: AMPH exposure does neither affect the release frequency nor the amount of DA released per vesicle as evidenced by amperometry in both PC12 and chromaffin cells. This is supported by the calcium data which do not show changes in $[Ca^{2+}]_i$.
Though the evidence for AMPH-mediated DAT reversal appears solid, the present findings argue for re-evaluation of the widely-accepted AMPH hypothesis with respect to vesicular DA leakage.

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NEUROTOXIC CONSEQUENCES OF WILSON'S DISEASE

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Purpose: The aim of the study was to evaluate the MR signs of brain intoxication in Wilson's disease (WD), the degree of brain lesions' resolution during the chronic course of chelating therapy, to correlate the delayed time of diagnosis with the degree of brain involvement and to suggest the explanation of the biochemical character of the lesions.

Methods: Brain MR examination was performed in 53 patients with WD (37 with neurologic and 16 with hepatic form) while brain MR reexamination was performed at 1.5T imager in 14 patients with neurologic form of WD, 3.5-7 years following the initial MR study.

Results: Four types of signal intensity (SI) changes were reported: 1) high SI on T2W images in basal ganglia, brain stem, cerebellar peduncles and supratentorial white matter 2) low SI on T2W images in globus pallidus, substantia nigra and red nucleus, compatible with iron deposition 3) high SI on T1W images in globi pallidi in patients with portosystemic shunt and 4) putaminal proton density signal elevation with no T1W or long-echo T2W abnormalities. Frank symmetry of the lesions was found. Complete reversal of putaminal T2W signal alterations was noted in 60% of patients with short (up to 18 months) interval to correct diagnosis while no improvement was evident in majority of patients with delay of 24 months and longer. Significant regression of brain stem lesions was present both in patients with short and long interval to accurate diagnosis. Long-term follow-up MR study revealed significant progression of ventricle dilation in 42.85% (6/14) of patients.

Conclusion: Patients with neurologic presentation of WD and short delay to correct diagnosis presented with more likely reversible putaminal signal alterations. The initial morphologic substrate of the symmetric T2W/PDW hyperintense lesions in the basal ganglia, brain stem and supratentorial white matter in WD appears not to be the deposition of copper but demyelination, possibly caused by generated toxic radicals due to impaired process of copper detoxification. This long term follow-up study indicates that appearance of new lesions with T2W/PDW signal elevation is arrested with continuous and adequate treatment. However the "normal" process of brain aging seems to remain accelerated. Putaminal myelinolysis, especially in patients with late recognition of WD, seems to be less likely reversible process, leading to gliosis and other end-stage neurodegenerative changes resistant to chelating therapy.

ASSESSMENT OF THE AXONOPATHIC POTENTIAL OF THE UNSATURATED BUTENENITRILES IN THE RAT

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Purpose. The unsaturated butenenitriles show diverse neurotoxic properties: allylnitrile and *cis*-crotononitrile cause degeneration of the auditory and vestibular hair cells, while *trans*-crotononitrile causes neuronal degeneration in discrete brain areas of the rat, including the inferior olive and the piriform cortex. The audiovestibular effect is shown also by 3,3'-iminodipropionitrile (IDPN), a nitrile known to cause also a proximal neurofilamentous axonopathy and degeneration in the visual and olfactory systems. This work assessed whether the unsaturated butenenitriles are axonopathic.

Methods and Results. Male Long-Evans rats received no nitrile, IDPN (positive control), allylnitrile, *cis*-crotononitrile or *trans*-crotononitrile (3.25, 0.89, 1.79 and 3.75 mmol/kg/day for 3 days, i.p., respectively), and were examined for presence of axonopathic lesions in the dorsal root ganglia at 8 days by light microscopy in semithin sections. IDPN animals showed axons with increased diameter; similar effects were not observed in allylnitrile or *cis*-crotononitrile animals. In one of the *trans*-crotononitrile animals, two giant axonal swellings (balloons) were observed. On transmission electron microscopy observation, the balloons were found to be filled up with neurofilaments. To pursue this finding, further animals were i.p. exposed to *trans*-crotononitrile at 3.25 mg/kg/day x 10 days, or via drinking water to 18, 36 and 72 mM, successively, for 3 weeks each. On histological examination, these animals did not show overt axonal enlargement in comparison to controls.

Conclusion. The axonopathic potency of allylnitrile, *cis*-crotononitrile and *trans*-crotononitrile is either null or significantly smaller than that of IDPN.

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IMPACT OF IPM TRAINING IN ILLINOIS CHILDCARE CENTERS

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Purpose: In July 2004, Illinois passed Senate Bill 1079 to regulate the use of pesticides in licensed daycare facilities, requiring them to develop and implement a formal IPM policy including management, pest control and pest prevention practices. The Chicago Safer Pest Control Project (SPCP) initiated the "Train the Trainer" program in 2003 to train childcare providers, Department of Children and Family Services (DCFS) supervisors, and Department of Health Services (DHS) nurses who in turn would train their staff and other childcare providers.

A follow-up survey of 3364 Illinois daycare centers was conducted in 2007, to assess the direct and indirect impact of formal Integrated Pest Management (IPM) training by SPCP and other organizations in Illinois. Babies and younger children are especially vulnerable to pesticide exposure as a result of behavioral and physiological factors. Children have immature organs, more unprotected skin and higher metabolic and respiration rates than adults, and exhibit mouthing activity with pesticide contaminated objects. Parents, childcare workers and staff are generally untrained in using pesticides and may not follow safety recommendations or consider safer alternatives in efforts to provide a sanitary pest-free environment.

Methods: The study used two survey instruments:
A. Pre Training Questionnaire
Part 1: Current pest problems and actions, administered at the start of the training session.
Part 2: Intent to implement selected IPM practices, administered at the end of the training session.
B. Post Training Questionnaire (assessment of IPM Practices)
1. Mailed to all formerly SPCP trained childcare centers
2. Mailed to all other Illinois licensed childcare centers (control group) that may have received training elsewhere.

Results: Of the 3364 post-training surveys mailed, 316 (9.4%) of the childcare centers responded; 99 of which were listed as formerly trained by SPCP and 69 of whom had completed both pre- and post- training assessments. Forty-nine of the 217 non-SPCP trained childcare centers (control group) reported little knowledge of IPM or the Illinois IPM law, while 168 respondents reported learning about IPM from DCFS, DHS nurses, their pest control contractor, brochures, internet and other sources. Childcare centers reporting training by SPCP or another source expressed a more positive view towards IPM then centers reporting no IPM training. SPCP training was associated with significantly greater understanding of the IPM law, reduction in pest infestations, and implementation of selected IPM practices and diffusion of IPM practices into staff homes. Understanding the IPM law was associated with working cooperatively with parents and the pest contractor. IPM training and regulations (know about and confident complying

with IPM law) was associated with reducing pesticide spraying, a key component of the law.

Conclusions: This study evaluated IPM knowledge and adoption in 316 childcare centers, responsible for the safety of approximately 27,424 children.

Results show a significant increase in understanding the IPM law and implementing IPM practices in childcare centers that received training, especially working closely with pest contractors and reducing pesticide spraying.

When SPCP trained centers were asked to specify new actions, less than 16% reported implementing IPM management or pesticide practices compared to 24% whom adopted preventative practices (better cleaning, reducing clutter, using door-sweeps, patching holes) – the cornerstone of effective IPM.

The study results and childcare provider comments suggest that after IPM training, childcare providers internalized the importance of IPM to 1) safeguard children and 2) comply with regulations; mostly through working cooperatively with pest contractors and implementing inexpensive housekeeping practices; and less by complying with formal management requirements.

NEUROLOGICAL ALTERATIONS FOLLOWING A SINGLE DOSE OF POLYBROMINATED DIPHENYLETHER 47 IN MICE ON POSTNATAL DAY 10

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Polybrominated diphenylethers (PBDEs) are commercial flame retardants that are accumulating in the environment. 2,2',4,4'-Brominated diphenylether (BDE 47), a stable PBDE congener, has been shown to accumulate in humans. Additionally, there is a growing literature of animal studies that show subtle changes in motor and cognitive function following acute or repeated perinatal exposure to PBDEs.

Purpose: The current study examined thyroid hormone levels, long-term behavioral outcomes, developmental milestones, and neurochemical status in mice following a single dose of BDE 47 during development.

Methods: C57BL/6 mice were administered BDE 47 (0, 1, 10, or 30 mg/kg p.o.) on postnatal day 10. Behavioral endpoints included maturation of sensory and motor function, and motor activity levels. Mice were sacrificed on selected days for analysis of: 1) total thyroxine (T4) and triiodothyronine (T3); 2) monoamines in cortex, striatum, and cerebellum; and, 3) D2 receptor and VMAT mRNA in the same brain regions.

Results: No effects were observed on T4 or T3 at any age examined. BDE 47 exposure increased body weight in adults. There was altered ontogeny in a few measures of neuromotor development. Motor activity was altered in adult mice, with BDE 47-treated mice (all doses) displaying pronounced hyperactivity. Male mice showed increased dopamine levels at all ages measured, but only in the middle dose group. On the other hand, female mice showed transient decreases in striatal dopamine, HVA, and DOPAC only at the lowest dose, and a persistent but small decrease in serotonin at the middle dose. There were no differences in D2 or VMAT expression.

Conclusions: These results suggest that: 1) a single dose of BDE 47 during postnatal development produces changes in the development of neuromotor systems and in adult behavior; 2) these neurological abnormalities are not due to acute changes in circulating thyroid hormones; and, 3) altered neurotransmitter levels in cortex and striatum may play a role in the neurobehavioral outcomes.

This is an abstract of a proposed presentation and does not reflect US EPA policy.

DIFFERENTIAL SPATIAL DISTRIBUTION OF PARKINSON'S DISEASE MORTALITY IN SPAIN − IS THERE A LINK WITH ENVIRONMENTAL EXPOSURE TO NEUROTOXIC XENOBIOTICS FROM PERSISTENT INDUSTRIAL POLLUTANTS?

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Purpose: Aetiologically, genetic and environmental factors having an uneven spatial distribution may underlie Parkinson's disease. The purpose of this study was to describe and analyse municipal mortality due to Parkinson's disease (PD) in Spain in an aetiological and interventional perspective.

Methods: PD mortality at a municipal level from 1989 to 1998 was modelled using the Besag-York-Mollié autoregressive spatial model, combining demographic information with cause-of-death diagnostic data (International Classification of Diseases 9th Revision (ICD-9) code 332.0). Municipal relative risks (RRs) were independently estimated for women, men and both sexes, and plotted on maps depicting smoothed RR estimates and the distribution of the posterior probability of RR>1.

Results: A south-north gradient, with large geographical areas suggesting clustered towns with high mortality, was seen in Asturias, the Basque Country, Balearic Islands and, particularly, in the Lower Ebro valley around Tarragona. Similarly, there was a suggestion that lowest mortality was clustered in the south-east and south-west. We also identified some isolated or clustered municipalities that were situated near industrial plants reported to be associated with environmental xenobiotic emissions.

Conclusions: Municipal PD mortality in Spain as seen from death records was unevenly distributed. Patterns were roughly similar to reported provincial PD mortality and use of levodopa. While it is possible that selected "hot spots" reflect genetic factors and/or environmental exposures inducing parkinsonism, the overall pattern may additionally result from case under ascertainment. However, along most areas of higher PD mortality there exists since decades a huge amount of emissions and waste of organochlorines and related compounds. A few municipal populations, located in low-mortality-risk areas in the vicinity of polluting plants or registering high excess PD mortality, might constitute a priority for conducting direct studies.

[CIBERESP: CIBER de Epidemiología y Salud Pública; CIBERNED: CIBER de Enfermedades Neurodegenerativas]

BRAIN REGIONAL AND TEMPORAL DYNAMICS OF ACHE AND ACYLPEPTIDE HYDROLASE AFTER ACUTE CHLORPYRIFOS

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Purpose: The organophosphate Chlorpyrifos has been related to different neurotoxic effects long after neonatal or adult exposure. In particular acute exposure of adult rats to a high dose affects spatial learning and memory tasks and increase impulsive response. Long term neurochemical changes have been reported also. The mechanisms responsible for these effects are not clear. Despite the well know inhibition of acetylcholinesterase enzyme (AChE), others biological targets have been proposed as responsible for the neurotoxic effects of chlorpyrifos and, perhaps, other organophosphates. Acylpeptide hydrolase (ACPH) has been proposed recently as a very sensitive enzyme to the exposure to organophosphates. The aim of the present work was to compare the distribution of AChE and ACPH activity in different brain areas and the time course of its inhibition after exposure to three acute doses of CPF.

Methods: Groups of 12 rats were ip injected with 50, 125 250 mg/kg of CPF or with 1ml/kl of olive oil. Sets of three animals per group were sacrified at 48h, 7, 15 or 30 days after intoxication. Brains were dissected in frontal cortex, hippocampus, amygdale, caudate-putamen, cerebellum and brain stem. AChE activity was determined by Ellman method; ACPH activity was determined as described previously by Perrier et al (2002).

Results: The three doses of chlorpyrifos inhibited both enzymes. AChE inhibition was more profound than that of ACPH for the highest dose of CPF, with the exception of the amygdala, where both enzymes were equally inhibited to almost no activity 48h after intoxication. Time course of recovery was similar for both AChE and ACPH, reaching 20-30% of control activity at 30 days post-exposure.

Conclusions: High acute doses of chlorpyrifos, able to inhibit more than 50% of AChE activity are shown to also inhibit ACPH activity in different rat brain areas.
To the extent that ACPH activity could be related to neurodegenerative proceses by its ability to metabolize beta-amyloid peptides, the present data could be regarded as a potential neurotoxic mechanism that deserve further investigation

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ESTIMATION OF CHEMICAL INJURY SEVERITY IN CASES OF MASS INHALATIONAL POISONINGS

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Background: Mass hospitalization in result of acute inhalational poisoning is actual issue in medical practice. There is carbon monoxide poisonings (*CO*) remain a serious problem of clinical toxicology. *CO* causes tissue hypoxia and produces various systemic and neurological complications, and according to some opinions, concentration of *COHb* ≥ 60% a lethal outcome ensues.

Purpose: Comparative study of the outcomes of mass acute inhalational poisoning and prognostic value of *COHb* concentration in cases of *CO*.

Materials: We carry out the retrospective study total of 523 medical cards patients with unintentional poisonings: 230 - chlorine, 31 – ammonia, 100 - polyisocyanate, and 162 - *CO* (mean age 42,1±1,2 years). The state of patients was assessed according to the scale introduced by Persson et al (1998).

Results: The study found a significant difference in *COHb* concentration between groups of exited and recovered patients in the first day (48,66±1,05 and 30,23±0,99, respectively; t=12,7; p<0,001). Corelational dependence between *COHb* concentration and the clinical assessment of the poisoning severity according to Persson et al. (1998) was r=+0,42 (p<0,05).

Conclusions: 1. Under our findings most of people die prior to hospitalization in result of acute inhalational poisoning. Notwithstanding the specialized medical treatment, mortality in the cases of chlorine poisonings reaches 8,3%. But, lethality was higher in group of carbon monoxide patients – 5,4%. 2. The study found a statistically significant difference in *COHb* concentration between groups of exited and recovered patients (P>0,001). 3. The study registered on the degree of severity of the poisoning depending an increase of *COHb* concentration. 4. Result of Persson's scale estimation of inhalational poisoning largely reflects the degree of neurological disorders.

ACUTE LOW DOSE METHYLMERCURY (MeHg) EXPOSURE ACTIVATES CASPASE-3 IN POSTNATAL RAT HIPPOCAMPUS

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MeHg, an environmentally persistent toxicant, induces a spectrum of harmful effects on neurodevelopment. Our recent studies (Falluel-Morel et al. 2007) have shown that a single sc injection of 5µg/g MeHg in 7 day old (P7) rats acutely activates caspase-3, an apoptotic protease, leading to cell cycle arrest and cell death in hippocampus. Furthermore, reduced cell number and organ size were detected at P21 as well as poor spatial learning and memory at P35. Caspase-3 activation, a marker for apoptosis, may involve either the extrinsic pathway that depends on caspase-8 or the intrinsic pathway (involving caspase-9), which we found to underlie hippocampal neurotoxicity (Sokolowski et al. 2008).

Purpose The purpose of this study is to use caspase-3, a significant endpoint marker, to define detrimental effects of lower doses of MeHg.

Method Based on the pathway definition, we used low doses of MeHg in our model of hippocampus toxicity, to approximate the effects of environmentally relevant exposures. Of all the molecular markers we studied, including [3H]-thymidine incorporation, BrdU labeling, total DNA content and absolute cell number, activated caspase-3 immunoreactivity was the most sensitive. After 24hr treatment with vehicle or MeHg (10, 0.6, 0.2, 0.1µg/g) in the P7 rat, whole brains were prepared for frozen sectioning and caspase-3 immunostaining.

Results MeHg exposure (0.2µg/g) induced a two-fold greater number of activated caspase-3-positive cells compared to controls. The next higher dose, 0.6µg/g MeHg, elicited a five-fold increase in caspase-3-positive cells while the highest dose, 10µg/g MeHg, increased positive cells eight times greater than control.

Conclusions By using the most sensitive marker for apoptosis, caspase-3 immunostaining, we now detect significant MeHg neurotoxicity at a 25-fold lower dose than previously defined. Future studies will characterize acute effects on hippocampal neurogenesis and later consequences on organ/cell composition and cognitive functions.

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ELECTROMAGNETIC FIELD IRRADIATION EMITTED FROM CELL PHONES AND ATTENTION DEFICIT- HYPERACTIVITY DISORDER IN CHILDREN

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Purpose: Recent studies have shown that EMF irradiation probably affects brain development. Pregnant woman who consistently use cellular phones are prone to deliver children with Attention Deficit Disorder (ADD) or Attention Deficit- Hyperactivity Disorder (ADHD), in significantly higher occurrence as compared to the general population. The previous study (Friedman et al, 2007) have demonstrated the biological effects might be caused by EMF irradiation to the human fibroblast cells by ERK phosphorylation cascade. Consequently, we have developed the substance SR18 composed of herbs and minerals in diluted form, which may effectively prevent the biological effect as shown *in vitro* in human fibroblast cells. These biological effects are caused by cell phone irradiation. In the present study we aimed to demonstrate the significant attenuation exerted by SR 18 on these effects and how it prevents the negative cascade reaction.

Methods: We checked the level of ERK phosphorilation produced by 5, 10, 15 and 20 minutes of cell phone irradiation in half of the regular intensity. Group 1 was the regular cells, the second group was the treated group with SR 18 in two forms: a form in which the liquid was given inside the cells and the second one the petri plate was surrounded by SR 18 in silicon tube. The third group was the unhandled control group. The same procedure was given to other cells in order to find the effects of the EMF irradiation on P 53 and on the same type of cells.

Results: complete reduction was observed in ERK phosphorylation secondary evolved to EMF irradiation. We also improved the P53 involvement. The SR 18 prevented the normal reaction of P 53 to this type of stress. By that way the cells could not be subjected to apoptosis.

Conclusions: Both ADD and ADHD may be partially related to P53; and SR18 substance may protect EMF irradiation neurobehavioral damage and this protective effect should be further investigated. Meanwhile, our suggestion is to reduce using cell phones to the minimum necessary.

EFFECTS OF PERINATAL HYPOTHYROIDISM ON AUDITORY STARTLE RESPONSES AND PREPULSE INHIBITION IN WEANLING AND ADULT RATS

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Purpose: Hypothyroidism is one of the risk factors that cause developmental disorders in children because thyroid hormone systems are essential for brain development. This study aimed to clarify whether hypothyroidism affects neurobehavioral developments in weanling and adult rats.

Methods: Pregnant rats were treated with methimazole (MMI) at concentrations of 0, 0.002, and 0.02% (w/v) in drinking water from gestational day 15 to postnatal day 21. Offspring were examined using prepulse inhibition (PPI) at the age of 1, 6, and 12 months. A 115-dB white noise with 40 ms duration was presented as a startle stimulus. A white noise stimulus of 75, 85, or 95 dB with 20 ms duration each preceded the startle stimulus as a prepulse for 30 ms. The percentage of PPI was calculated as following: %PPI=100×(P-PP)/P where P or PP are the average startle amplitudes under the startle stimulus alone or startle stimulus preceded by a prepulse. This research was approved by the Center for Advanced Science and Technology (Hokkaido University). All conditions complied with the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

Results and Conclusions: The 0.02%-treated group decreased %PPI compared to both the control and 0.002%-treated groups at the age of 6 and 12 months. This reduction was obtained when the prepulses with 75 dB and 85 dB intensities were preceded. These findings suggest that perinatal hypothyroidism gives rise to hearing deficit to these prepulses with low intensities because the MMI doses did not affect %PPI when the prepulse with 95 dB intensity was presented. However, the 0.02%-treated group displayed greater amplitudes of startle responses compared to both the control and 0.002%-treated groups. The greater reaction to startle stimuli may interfere with learning or cognition.

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IN VITRO NEUROTOXICITY OF BROMINATED FLAME RETARDANTS STUDIED IN PC12 CELLS

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Purpose: Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), are used in a wide range of consumer products. Though these environmental contaminants were initially identified as endocrine disruptors, it has been shown that neonatal exposure to BFRs induces neurobehavioral changes[1,2] and can reduce long-term potentiation (LTP) in mouse hippocampal slices[3]. Additionally, BFRs have been shown to affect the intracellular calcium concentration ($[Ca^{2+}]_i$) *in vitro*[2,3].
The aim of the current study was to determine the effects of the environmentally relevant BDE-47, its hydroxylated metabolite (6-OH-PBDE-47) and a possible alternative additive flame retardant (HBCD) on $[Ca^{2+}]_i$ and exocytosis using PC12 cells as neuroendocrine *in vitro* model.

Methods: Exocytosis was recorded using amperometry[4] and changes in $[Ca^{2+}]_i$ were determined using the calcium-sensitive fluorescent ratio dye Fura-2 in PC12 cells.

Results: Exposure of PC12 cells to 6-OH-PBDE-47 ($\geq$1 µM) induced a biphasic increase in $[Ca^{2+}]_i$, whereas BDE-47 ($\leq$5 µM) was ineffective. The increase in $[Ca^{2+}]_i$, which was accompanied by an increase in exocytosis, was induced by Ca^{2+} release from endoplasmic reticulum and mitochondria.
Exposure of PC12 cells to HBCD (0.2-20 µM) did not affect basal $[Ca^{2+}]_i$ or the basal frequency of exocytosis. However, the number of cells showing depolarization-evoked exocytosis was markedly reduced. Subsequent experiments revealed that HBCD caused a dose-dependent reduction of the depolarization-evoked increase in $[Ca^{2+}]_i$ via a-specific inhibition of L-, N- and P/Q-type voltage-gated Ca^{2+} channels.

Conclusions: The present study demonstrates that 6-OH-PBDE-47 is more potent in disturbing Ca^{2+} homeostasis and inducing exocytosis than its parent compound BDE-47. It can thus be concluded that bioactivation by oxidative metabolism increases the neurotoxic potential of PBDEs. HBCD, a probable replacement candidate for PBDEs, has a different mechanism of action, i.e., inhibition of depolarization-evoked $[Ca^{2+}]_i$ and exocytosis.
It is not unlikely that future human exposure to BFRs increases, justifying additional efforts to establish an adequate exposure, hazard and risk assessment. PC12 cells will prove to be a valuable *in vitro* model for hazard characterization of these and other persistent environmental contaminants.

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ATTENUATION OF ARSENIC NEUROTOXICITY BY CURCUMIN IN RATS

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Presence of arsenic as a contaminant in ground water in some regions in India and several other countries is a serious risk to humans. Use of arsenic in industries and mining has significantly contributed to enhance its levels in the environment. In view of the continued exposure to arsenic and associated human health risk including neurotoxicity, studies have been carried out to investigate neuroprotective efficacy of curcumin. Rats treated with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) exhibited a significant decrease in locomotor activity (distance traveled 48%, time movement 43%), grip strength (26%) and rota-rod performance (82%) in comparison to controls. A decrease in the binding of 3H-Spiperone to striatal membrane (32%), known to label dopamine – D2 receptors and tyrosine hydroxylase (TH) immunoreactivity in striatum was observed in arsenic treated rats. An increase in arsenic levels in frontal cortex (6.3 fold), corpus striatum (6.5 fold) and hippocampus (7.0 fold) was observed in rats treated with sodium arsenite compared to controls. Exposure to sodium arsenite in rats caused an increase in the level of lipid peroxidation and protein carbonyl and a decrease in reduced glutathione in frontal cortex, corpus striatum and hippocampus as compared to controls. Activity of superoxide dismutase, catalase and glutathione peroxidase was found decreased in these brain regions in arsenic treated rats suggesting increased oxidative stress. Simultaneous treatment with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) and curcumin (100 mg/kg, p.o., 28 days) in rats caused an increase in locomotor activity (distance traveled 38%, time movement 32%), grip strength (20%) and improved the rota-rod performance (76%) as compared to those treated with arsenic alone. An increase in the binding of striatal dopamine receptors (17%) and TH expression was also observed in these rats as compared to those treated with arsenic alone. Arsenic levels in frontal cortex (2.3 fold), corpus striatum (1.2 fold) and hippocampus (1.6 fold) were found to be decreased in rats simultaneously treated with arsenic and curcumin. A decrease in the levels of lipid peroxidation and protein carbonyl and an increase in reduced glutathione levels were observed in corpus striatum, frontal cortex and hippocampus in these rats as compared to those treated with arsenic alone. Interestingly, activity of superoxide dismutase, catalase and glutathione peroxidase was found to be increased in rats treated with arsenic and curcumin simultaneously. No significant effect on any of the above parameters was observed in rats treated with curcumin as compared to controls. A significant protection in behavioral, biochemical and immunohistochemical parameters in rats simultaneously treated with arsenic and curcumin, observed in the present study suggest the neuroprotective efficacy of curcumin.

UPREGULATION OF THE HETERODIMERIC AMINO ACID TRANSPORTER y^+LAT2 IN BRAIN IN HYPERAMMONEMIA: A LINK TO IMPAIRED MODULATION OF THE NO/cGMP PATHWAY BY GLUTAMINE?

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Purpose: Ammonia is a main pathogenic factor in neurological disorders associated with hyperammonemia (HA). One of the immediate effects of ammonia toxicity is an increased activity of NO/cGMP pathway and L-glutamine (Gln) accumulation in the brain. Pharmacological manipulations elevating extracellular Gln inhibit the NO/cGMP pathway in control rat brain and alleviate its stimulation following acute intracerebral infusion of ammonia. This effect was prevented by administration of L-arginine (Arg) and mimicked by leucine and cyclo-leucine, indicating involvement of the y^+LAT2 system counter-transporting Gln and Arg (Zielińska et al, Neurobiology of Disease, in revision). This study tested the effect of prolonged exposure to ammonia on i) modulation of the NO/cGMP pathway by Gln and ii) y^+LAT2 expression and activity.

Methods: Adult male Sprague - Dawley rats were used and HA was induced by 3 i.p. injections of ammonium acetate (600 mg per kg) at 24h intervals. Isolated cerebral cortex (cc) for mRNA expression of y^+LAT2 (Real time –PCR) and cc slices for transport experiments with [^{3}H] radiolabeld Arg and Gln were used.

Results: Prolonged exposure of rats to ammonia abolished the ability of exogenously added Gln to inhibit the NO/cGMP pathway and specifically increased the expression of y^+LAT2 mRNA in cc, and altered parameters of Gln and Arg uptake and/or exchange in a manner consistent with increased y^+LAT2 activity in cc slices: it increased i) the sensitivity of Gln uptake to Arg, and of the sodium-dependent component of Gln uptake to Glu, and ii) the sodium-dependent Arg uptake and its sensitivity to N-ethyl-maleimide, inhibitor of cationic amino acid transport.

Conclusions: Prolonged exposure to ammonia upregulates the expression and activity of y^+LAT2. An enhanced operation of Gln/Arg exchange by y^+LAT2 may be partly responsible for the here reported absence of response of the NO/cGMP pathway to Gln in cortical tissue slices derived from HA rats.

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