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Genetic aspects of behavioral neurotoxicology

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ABSTRACT

Considerable progress has been made over the past couple of decades concerning the molecular bases of neurobehavioral function and dysfunction. The field of neurobehavioral genetics is becoming mature. Genetic factors contributing to neurologic diseases such as Alzheimer's disease have been found and evidence for genetic factors contributing to other diseases such as schizophrenia and autism are likely. This genetic approach can also benefit the field of behavioral neurotoxicology. It is clear that there is substantial heterogeneity of response with behavioral impairments resulting from neurotoxicants. Many factors contribute to differential sensitivity, but it is likely that genetic variability plays a prominent role. Important discoveries concerning genetics and behavioral neurotoxicity are being made on a broad front from work with invertebrate and piscine mutant models to classic mouse knockout models and human epidemiologic studies of polymorphisms. Discovering genetic factors of susceptibility to neurobehavioral toxicity not only helps identify those at special risk, it also advances our understanding of the mechanisms by which toxicants impair neurobehavioral function in the larger population. This symposium organized by Edward Levin and Annette Kirshner, brought together researchers from the laboratories of Michael Aschner, Douglas Ruden, Ulrike Heberlein, Edward Levin and Kathleen Welsh-Bohmer conducting studies with *Caenorhabditis elegans*, *Drosophila*, fish, rodents and humans studies to determine the role of genetic factors in susceptibility to behavioral impairment from neurotoxic exposure.

1. Introduction

This symposium organized by Edward Levin and Annette Kirshner brought together researchers pursuing *Caenorhabditis elegans*, *Drosophila*, fish, rodent and human studies to determine the role of genetic factors in susceptibility to behavioral impairment from neurotoxic exposure studies. The laboratories of Drs. Michael Aschner, Douglas Ruden, Ulrike Heberlein, Edward Levin and Kathleen Welsh-Bohmer all contributed to this work.

Behavioral neurotoxicology has been instrumental in identifying and characterizing the functional consequences of neurotoxicants on the function of both experimental animals and humans. In the past, the search for mechanisms of behavioral toxicity focused mainly on neurochemical and neuropathological studies. Advances in molecular neurobiology have opened the way for determining genomic mechanisms of behavioral neurotoxicology. The availability of a variety of technological and methodological advances to aid in the development of appropriate animal models and systems will do much to move the field of behavioral neurotoxicology forward. Experts in behavioral genetics and behavioral neurotoxicology need to inform each other of the potential use that new methods, technologies and tools could have on the field of behavioral neurotoxicology in establishing a more mechanistic basis for the role of neurotoxic exposure in behavioral dysfunction.

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Considerable progress has been made over the past couple of decades concerning the molecular bases of neurobehavioral function and dysfunction. The field of neurobehavioral genetics is becoming mature. Genetic factors contributing to neurologic diseases such as Alzheimer's disease have been found and evidence for genetic factors contributing to other diseases such as schizophrenia and autism are likely. This genetic approach can also benefit the field of behavioral neurotoxicology. There is great potential to rapidly advance the field of behavioral neurotoxicology by accessing the tools of molecular biology both for discovery of mechanisms and for better understanding of individual differences in neurotoxic response. It is clear that there is substantial heterogeneity of response concerning the behavioral impairments from neurotoxicant exposure. Many factors contribute to differential sensitivity, but it is likely that genetic variability plays a prominent role. Important discoveries concerning genetics and behavioral neurotoxicity are being made on a broad front from work with invertebrate and fish mutant models to classic mouse knockout models and human epidemiologic studies of polymorphisms. Discovering genetic factors of susceptibility to neurobehavioral toxicity not only helps identify those at special risk, it also advances our understanding of the mechanisms by which toxicants impair neurobehavioral function in the larger population.

A symposium on the genetic aspects of behavioral neurotoxicology was conducted at the International Neurotoxicology Conference in Rochester, NY, October 13–17, 2008. The goal of the symposium was to highlight the advantage of diverse animal models to elucidate the genetic basis of behavioral neurotoxicology. Each of the presenters highlighted the benefits of their model in studying aspects of genetic alteration on behavior.

Dr. Aschner presented the utility of *C. elegans* in studying mechanisms of toxic effects on the nervous system. He cited the advantages of *C. elegans*, its small size (adults are ~1 mm long), ease of maintenance, speedy generation time (3 days), and large brood size (>300 progeny per hermaphrodite) for its use in cellular, molecular, and genetic analyses. In addition, its genome and biosynthetic and metabolic pathways are highly conserved with mammals. Additional advantages are the ability to assess, among other endpoints, the behavior and genetic effects from a variety of exposures including metals and pesticides. One can also model various human neurodegenerative disorders in *C. elegans* using either mutant strains or chemical exposure. *C. elegans* also lends itself to modern technological approaches such as high-throughput analysis, microfluidics, and quantitative trait locus mapping to identify relevant genes and behaviors.

Dr. Ruden presented the merits of studying the genetics and genomics of neurotoxicology in *Drosophila melanogaster* (*D. melanogaster*). There are thousands of wild type and mutant strains available in which hundreds of developmental and behavioral mutations have been identified along with homologous genes for over 70% of known human disease genes. This availability allows for very sophisticated techniques to knockout, reduce or overexpress almost any gene in the genome. In addition, quantitative genetics will permit in *D. melanogaster*, deep sequencing, a newer analytic technology which can characterize individually rare polymorphisms. This will allow the study of the role of normal genetic variation in phenotypic differences, the technology of which eventually can be used to identify a quantitative trait (drug response) useful in "personalized medicine". Lastly, fruit flies are useful in identifying genes that may be resistant or sensitive to a toxicant exposure (Gene × Environment or G × E interaction) using a technique that he calls "genetical toxicogenomics" which may have major implications in the role of natural variation in nervous system development in a toxic environment.

Dr. Heberlein expanded on the usefulness of the *Drosophila* model in which to study the genes and pathways that mediate acute and chronic behavioral responses to environmental exposure, in this case ethanol. She pointed out that multiple hypotheses have been presented to explain ethanol-induced brain damage. The mechanisms proposed vary from the consequences of thiamine deficiency to the production of reactive oxygen species (ROS) and increased production of polyamines depending on cell type and developmental stage to explain the types of damage induced. Finally, ethanol is known to bind to *N*-methyl-D-aspartate (NMDA) and it is thought that this interaction may explain many of the drug's neurotoxic effects. Using flies, they have shown that acute ethanol exposure leads to widespread cell death in the antennae, the primary olfactory organs of flies. Ethanol-induced death of *Drosophila* olfactory neurons is apoptotic in nature, requires *shaggy* (*sgg*), the *Drosophila* homolog of GSK-3β, can be prevented by treatment with the GSK-3β inhibitor LiCl, and can be blocked by electrical silencing of the olfactory neurons, demonstrating that ethanol-induced death in these cells is due to excitotoxicity, requires NMDA receptors in the olfactory neurons, and that *sgg* and the NMDA receptor are likely acting in concert to mediate this effect. They hope to use their model for ethanol-induced neuronal cell death to identify genes and mutations involved in sensitivity to ethanol neurotoxicity allowing a greater understanding of the molecular processes of neuronal death, which is seen in alcoholic dementia.

Dr. Levin and co-workers have used zebrafish and rodent models to investigate the behavioral neurotoxicology of environmental toxicants. Primarily, they have concentrated on toxic effects on cognitive function and other aspects of behavioral plasticity. Zebrafish is the piscine model most widely used to study the molecular bases of development in general and neurodevelopment in particular. Their clear chorion and reporter systems allow continuous visualization of developmental processes. The variety of mutant models and the availability of morpholinos in which parts of the genome can be reversibility suppressed during early development provide ways to test the role of genetic factors in neurodevelopment. The Levin lab and others have developed a variety of behavioral tests to provide assessment of the functional consequences of neural impairment. Their behavioral tests assessing spatial learning and memory detected the persisting impairment caused by early developmental exposure to low doses of the pesticide chlorpyrifos. Chlorpyrifos also caused significant hyperactivity in a rapid test of motor reaction to a tactile startle. Chlorpyrifos-induced behavioral impairment have been related to alterations in neurochemical indices of dopamine and serotonin neurotransmitter systems in zebrafish.

Levin et al. have also worked with the classic mouse knockout model for testing genetic influences on behavior. In particular they have used metallothionein 1 and 2 knockout mice and tested the interactions with developmental exposure to mercury. Metallothionein 1 and 2 knockouts themselves have cognitive impairment. They also potentiate the persisting learning impairment caused by early postnatal mercury exposure at a dose that does not affect wild-type control mice. Metallothionein × mercury interactions in dopamine levels that were detected may be important in explaining the differential response to mercury in terms of cognitive function.

Dr. Welsh-Bohmer discussed the possible gene × environment interaction in Alzheimer's disease (AD) in human studies. While one of the most influential risk factors for AD is inherited disease susceptibility factors, genes may not be solely responsible for AD risk and symptom onset in most cases of AD. Some of the more speculative risk factors for AD are environmental toxicants, such as pesticides, organic solvents, air-borne pollutants, and heavy metals, have been linked to a number of neurological disorders including Parkinson's disease and multiple sclerosis. Since few studies have been conducted as to these neurotoxicants'