Session 4: Models of Neurobehavior and Neurotoxicology

Assessing the Subtle Neurological Effects of Environmentally Relevant Methylmercury Exposures in Zebrafish

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Environmental toxicants can have dramatic overt effects on the developing nervous system that are readily observed in the zebrafish. These might include individuals that remain nearly motionless or individuals that are nonresponsive to normal startle-inducing visual, vibrational, or mechanical stimuli. These overt effects normally occur at concentrations well in excess of those that are considered environmentally relevant. Our laboratory has been evaluating the effects of developmental exposure to low to moderate levels of neurotoxicants to link changes in molecular pathways, as revealed by transcriptomics, to behavioral deficits. We will discuss methylmercury as an example neurotoxicant that does not produce overt behavioral effects at low to moderate levels. Developmental exposure to environmentally relevant levels of methylmercury causes a wide variety of subtle neurobehavioral deficits in the young zebrafish that persist into adulthood, some of which are inherited for generations. These deficits include altered behavioral response to visual stimuli, changes in retinal electrophysiology, hyperactivity and learning deficits. We are working to develop higher throughput assays for assessing neurological function and single cell-type assays to explore the mechanism of neurotoxicity and of transgenerational inheritance in greater detail.

Transgenic Zebrafish Models for the Study of Dopamine Neuron Development, Loss, and Regeneration

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To facilitate studies of dopaminergic (DA) neuron development, loss, and potential regeneration, we have produced lines of transgenic zebrafish, in which transgenes are inserted in-frame within the first exon of the *dopamine transporter* (*dat*) gene, in order to target their expression to DA neurons. Transgenic lines include Tg(*dat:EGFP*), Tg(*dat*:*tom20MLS-mCherry*) and Tg(*dat-CFP-NTR*). In Tg(*dat:EGFP*) fish, DA neurons are labeled, including those in the ventral diencephalon (vDC) clusters, amacrine cells in the retina, in the olfactory bulb, in the pretectum, and in the caudal hypothalamus. In the vDC, DA neurons of groups 2–6 are correctly labeled. In Tg(*dat*:*tom20MLS-mCherry*) transgenic fish, a fusion of mCherry with the mitochondrial localizing signal of Tom20 targets the fluorescent reporter to mitochondria of DA neurons. Treatment of Tg(*dat*:*tom20MLS-mCherry*) larvae with the dopaminergic neurotoxin MPTP at either 2 or 3 days postfertilization results in a visible decrease in fluorescence of mCherry positive dopamine neurons compared to untreated larvae. In Tg(*dat-CFP-NTR*) fish, the cell-specific expression of nitroreductase results selective ablation of DA neurons when fish are treated with metronidazole (Mtz) from 36 to 120 hours postfertilization. Recovery of DA neurons is markedly enhanced by treatment with DA receptor agonists such as quinpirole. These transgenic lines are useful for the study of DA neuron development, as models of DA neuron loss and regeneration, and in chemical screens for compounds that influence DA neuron survival.

Functional Assays and Alternative Species: Using Larval Zebrafish in Developmental Neurotoxicity Screening

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The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals for developmental toxicity. We are exploring methods to detect developmentally neurotoxic chemicals using zebrafish behavior at 6 days of age by designing a behavioral testing paradigm capable of assessing the effects of sublethal and subteratogenic concentrations of developmental neurotoxicants. The behavioral paradigm simultaneously tests individual larval zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and intensity of light, we are able to assess changes in locomotion during light–dark transitions and adaptation to both light and dark during approximately 1 hour of testing. The testing format allows evaluation of large numbers of larvae, chemicals and chemical concentrations. Using this paradigm we have tested a training set of chemicals that are either known or generally considered positive or negative controls for producing developmental neurotoxicity in mammals. We have found that many developmentally neurotoxic compounds perturb behavior at subteratogenic doses, while many developmentally nonneurotoxic compounds do not perturb behavior. Exposure to developmental neurotoxicants may alter the overall level of activity in light and dark conditions or the pattern of activity. Therefore, results from the training set indicate that careful behavioral evaluation of zebrafish larvae may be able to identify mammalian developmental neurotoxicants.

Zebrafish as a Tool for Rapid, *In Vivo* Detection of Small Molecule Effects on the Vertebrate Brain

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Zebrafish larvae are vertebrates exhibiting complex neurophysiology that is remarkably similar to humans, yet they are also well suited for whole-organism, high-throughput behavioral screens. We have identified several reproducible larval behaviors amenable to rapid high-throughput screening in a 96-well format. These simple, inexpensive behavioral assays can be used to identify the potential of a candidate small molecule to transit the vertebrate blood–brain barrier and affect neurological processes within the vertebrate brain. Using computational approaches, we are able to compare the behavioral effect profiles of candidate small molecules to the behavioral profiles of human drugs and toxins, including those with well-defined modes of action. These comparisons provide us with clues concerning the therapeutic or hazard pathways perturbed by the novel compounds. Due to the availability of zebrafish genetic information, transgenic resources and molecular tools for genome manipulation, we are can then use these small vertebrate fish to test, *in vivo*, our newly generated hypotheses about the neurologic substrates and pathways targeted by the novel compounds.

Studying Parkinson's Disease-Related Environmental Toxins Using Zebrafish

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic (DA) cell loss and alpha-synuclein pathology in both the central and peripheral nervous systems. Mutations and polymorphisms in several genes have provided insight into the pathogenesis of PD but genetic influences can only account for a small fraction of all PD cases. We have been utilizing zebrafish to help identify environmental factors that lead to DA cell loss and synuclein pathology and to determine the mechanisms by which they act. Data will be presented that demonstrate that pesticides associated with an increased risk of developing PD are toxic to DA neurons in a synuclein-dependent manner. We have found that some of these act through proteasome (E1 ligase) inhibition and others through aldehyde dehydrogenase inhibition. We will also present data on our studies utilizing zebrafish to test potential disease modifying drugs.