

Molecular RESEARCH PROJECT Mechanisms

How and when does pesticide exposure alter brain development?
 What role do specific alterations in molecular and cellular pathways play in mediating pesticide effects on the developing brain?
 Do pesticides produce oxidative stress and does this contribute to their potential to cause neurodevelopmental toxicity?

PROJECT OVERVIEW

The Molecular Mechanisms Research Project evaluates the molecular and cellular mechanisms through which specific pesticides cause neurodevelopmental toxicity. Modern and historically used pesticides such as chlorpyrifos, arsenic and methylmercury will be examined for their capacity to alter the regulatory dynamics of neural cell proliferation, differentiation and cell death. Special emphasis will be placed on the role of cell-signaling pathways and oxidative stress as well as antioxidant defense mechanisms that can mitigate the toxicity associated with a particular toxicant.

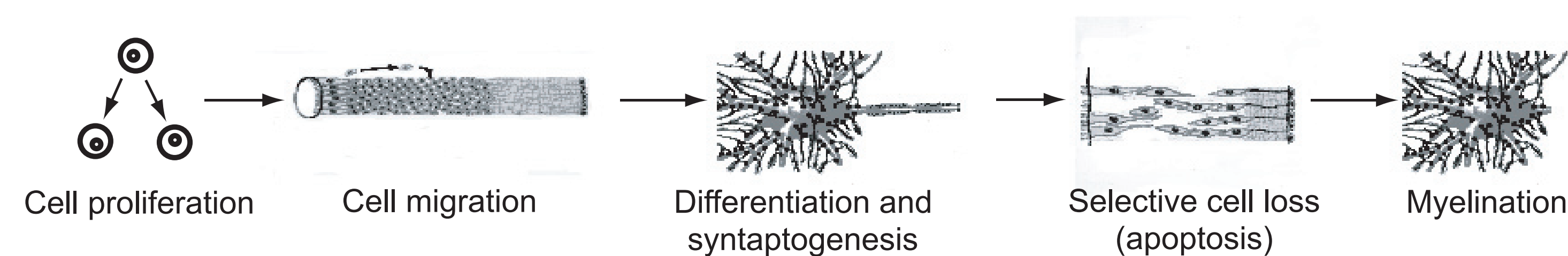


Figure 1. Illustration of brain developmental processes. This figure shows neurodevelopmental stages from neuroepithelial cell proliferation through processes of neuronal maturation (cell migration, differentiation, cell loss and myelination) during normal brain development.

HYPOTHESIS & SPECIFIC AIMS

Normal brain development involves a balance between cell proliferation, differentiation, and cell death, as shown in Figure 1. Our hypothesis is that exposure to certain pesticides can alter brain development and behavior by interfering with the regulatory pathways controlling these processes. Figure 2 illustrates the activity of these processes in specific brain regions across rodent development. It is important to note that each brain region has its own "signature." For example, in the developing midbrain proliferation and migration processes occur early in development and apoptosis later at post-natal times. In contrast, cerebellum has proliferation, differentiation and cell death occurring at the same time after birth. The timing and co-occurrence of these processes differs across species. In our research project, these processes are hypothesized to define "critical windows of susceptibility" to pesticide neurodevelopmental toxicity. Hence in this research project we are examining pesticide-induced alterations in the regulatory dynamics of normal cell proliferation, differentiation and cell death which result in altered CNS formation and differentiation of tissues and organs. These alterations are correlated with subsequent deficits in learning and development. We have designed studies to evaluate the commonality of these mechanisms of neurodevelopmental toxicity across a variety of chemical classes to test the similarity of these mechanisms of toxicity across agent, dose, life stage and cell type (neuronal versus glial cells) in order to more fully evaluate implications of these molecular observations for identifying agents with common modes of action.

Our focus on defining "windows of susceptibility" for many neurodevelopmental toxicants as well as for pesticides means the studies are relevant for a very broad range of critical environmental agents that can adversely affect neurodevelopment in children. Mechanistic information is missing for most agents, therefore both scientific and risk management approaches can be irregular and frequently inconsistent. This project evaluates mechanistic processes across pesticide classes in order to understand the commonality and uniqueness of pesticide toxicity across dose, life stage, cell type and behavioral domain.

To evaluate our hypotheses, we propose the following specific aims:

- Evaluate the effects and mechanisms of action by which pesticides affect neurogenesis versus gliogenesis.
- Evaluate the contribution of specific cell cycle control checkpoint pathways in defining susceptibility of neurons and glial cells to pesticide induced effects on proliferation.
- Evaluate the role that specific stress-activated cell signaling pathways (p38 MAPK, SAPK/JNK) play in mediating pesticide induced effects on viability, proliferation, and apoptosis in neuronal and glial cells.
- Evaluate the role that reactive oxygen species (ROS) generation and antioxidant machinery play in defining susceptibility to pesticide-induced neurodevelopmental toxicity.

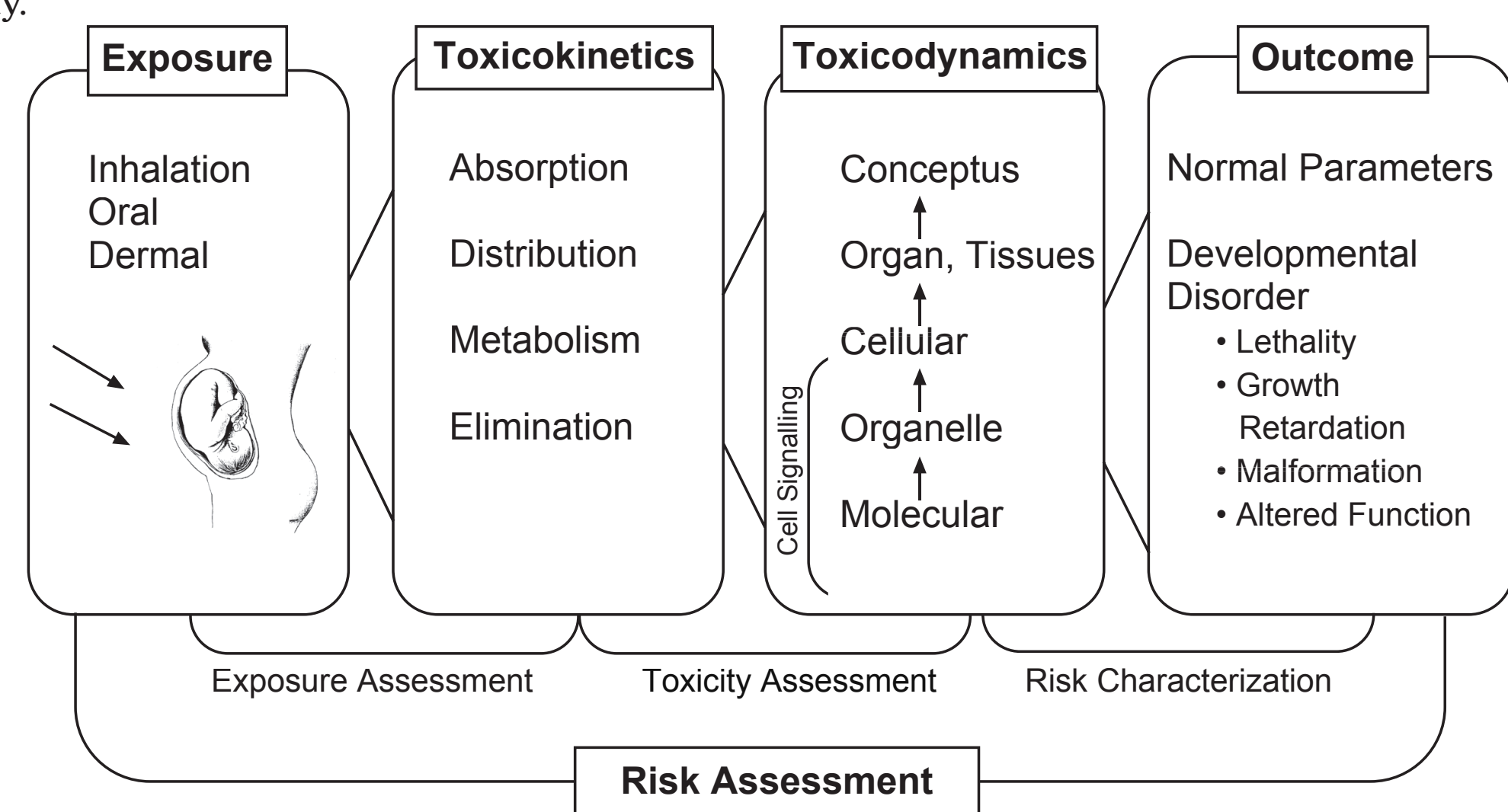
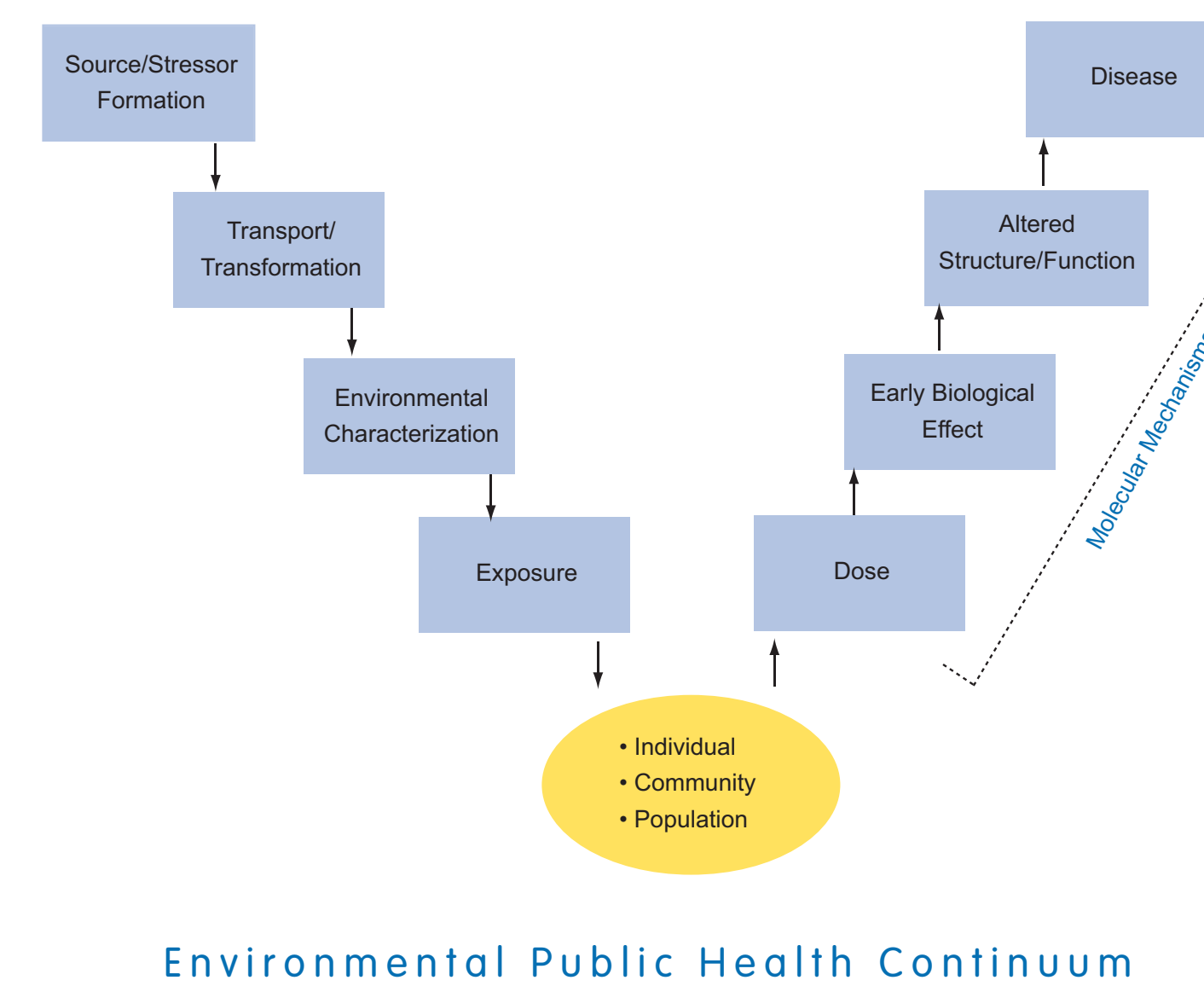


Figure 3. Framework to describe assessment of the effects of pesticides on development. The Molecular Mechanisms Research Project evaluates toxicodynamic changes in brain development.



ANTICIPATED OUTCOMES

- We will provide data showing the differential impact of chlorpyrifos, chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol on cytotoxicity, differentiation and apoptosis in embryonic midbrain neuronal cells. We hope to demonstrate that chlorpyrifos-oxon and TCP cause specific changes in apoptosis and may be more potent in inducing apoptosis than the parent compound.
- We will continue to incorporate our molecular findings in the biologically based dose response models for developmental toxicity that are being developed by the Risk Characterization Facility Core. For example, we have developed toxicokinetic and dynamic models for neurodevelopmental toxicity (Faustman et al. 2004) and a biologically based toxicodynamic model for midbrain and neocortical development with exposures to ethanol (Gohlke et al. 2002 and 2004) and methylmercury (Lewandowski et al. 2003), respectively. Figure 3 shows a framework which describes how the toxicodynamic impacts of pesticides are linked with overall changes in brain development and function. It also shows how exposure information is linked with these assessments.
- In the next year we will be examining the correlation between the level of chlorpyrifos-induced alteration in cell proliferation and/or death at critical times in the developing CNS and quantifiable alterations in postnatal development/behavior.

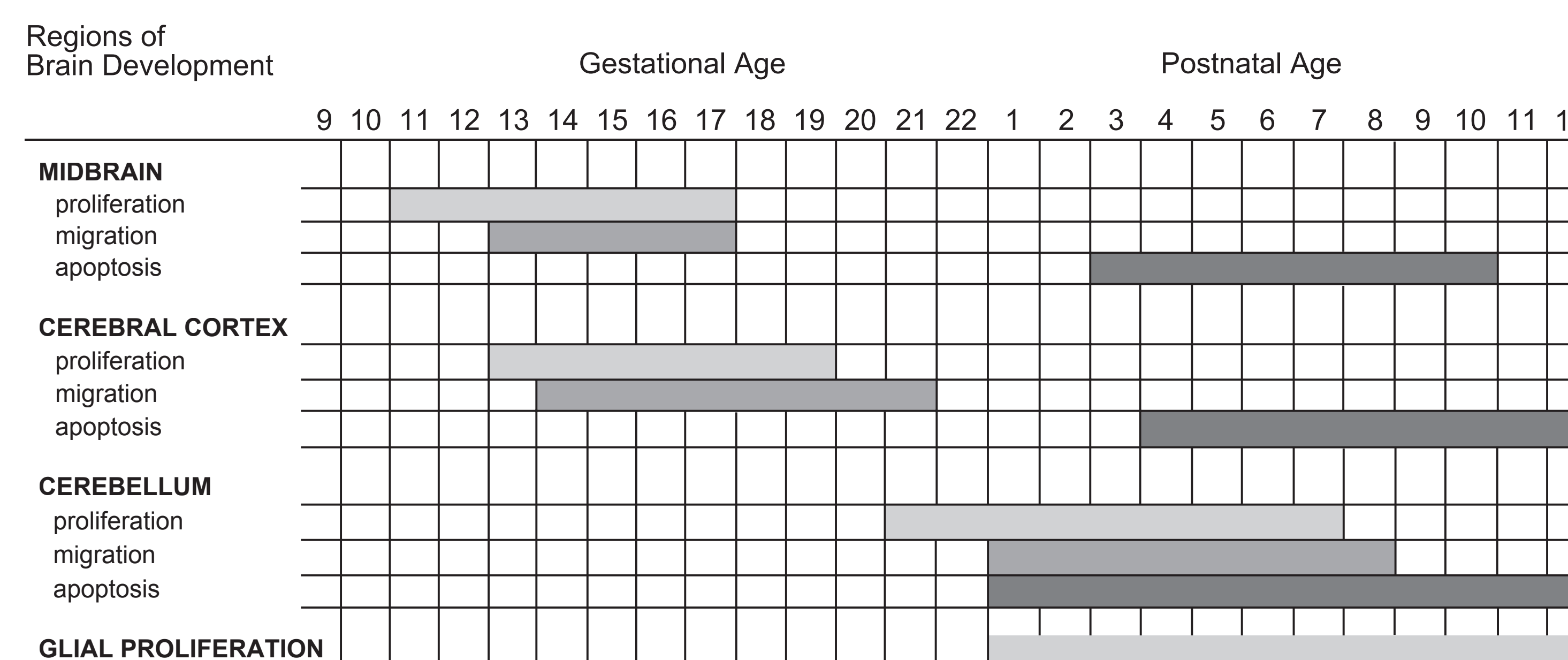
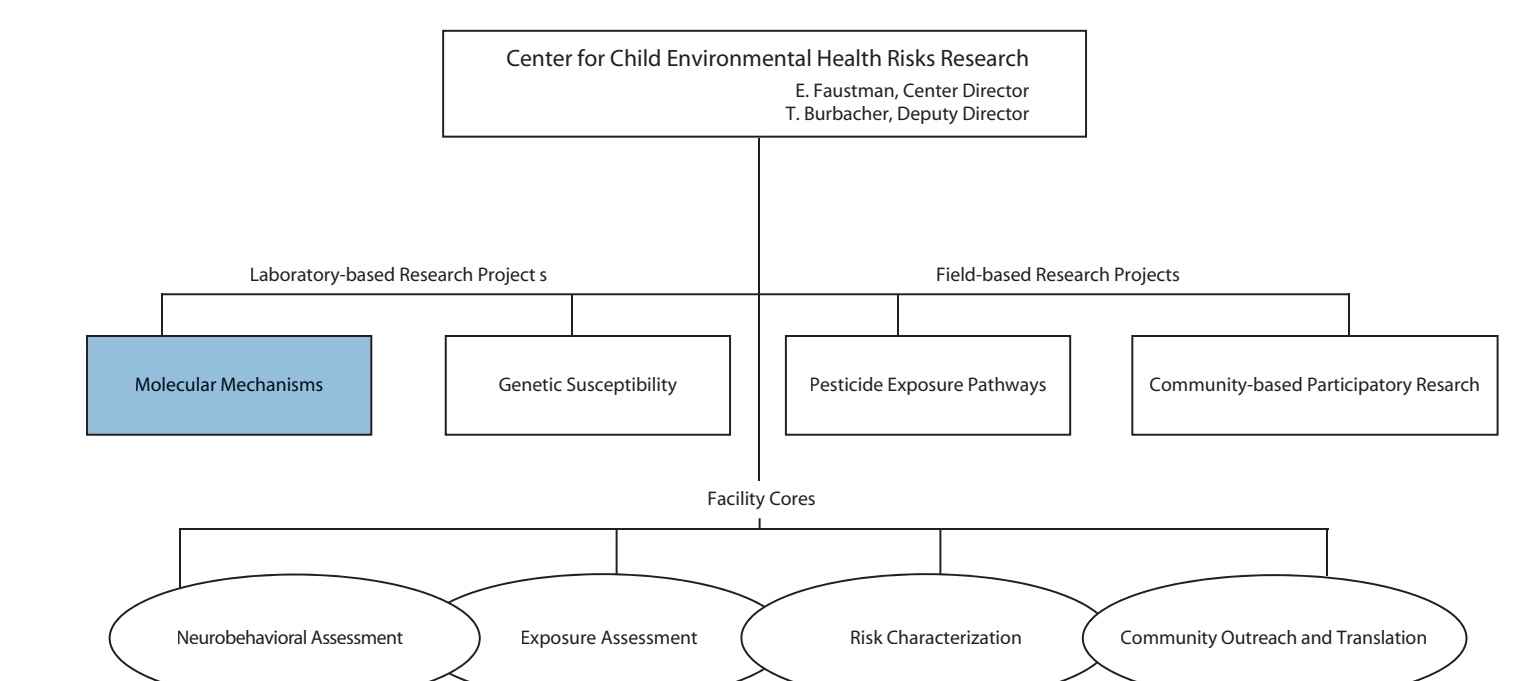


Figure 2. Laboratory studies focused on three brain regions assessing pre- and post-natal exposures.

RESEARCHERS



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