Vulnerability of synapses in the frontal cortex of mice developmentally exposed to an insecticide: Potential contribution to neuropsychiatric disease

W. Michael Caudle¹,²

¹Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322-3090
²Center for Neurodegenerative Disease, School of Medicine, Emory University, Atlanta, Georgia 30322-3090

Correspondence: W. Michael Caudle
E-mail: mike.caudle@gmail.com
Received: January 07, 2015
Published online: February 12, 2015

Increasingly, exposure to various chemicals found in our environment has been found to be a significant contributor to the risk of developing neurological disease, such as Parkinson disease, autism spectrum disorder, as well as other deficits in thought and function. Exposure to these compounds during critical periods of neurodevelopment, encompassing exposures that occur in utero, during infancy, childhood, and adolescence, represents a time period of nervous system growth that is uniquely vulnerable to disruption by environmental chemicals. Indeed, a contemporary hypothesis suggests that the pathological cascade associated with many common neurological disorders has its origin in disturbances of normal neurodevelopment. Moreover, alterations to the ontogeny of the synapse and neurotransmitter signaling during neurodevelopment may be a premier pathological event that underlies neuropsychiatric and neurodegenerative disease. To interrogate the impact of exposure to a ubiquitous environmental chemical, the pesticide, endosulfan, on development of neurotransmitter circuits, we coupled in vitro and in vivo platforms to evaluate its effect on the formation of GABAergic, glutamatergic, and dopaminergic pathways in the frontal cortex. With this approach we found exposure of cortical neurons, in vitro, exhibited a marked reduction in the length of their neurite process as well as the number of synaptic connections. Further investigation using an in vivo model of developmental exposure identified significant alterations to pre and postsynaptic proteins involved in neurotransmitter handling and signaling in each of the neurotransmitter systems investigated. These findings suggest that exposure to endosulfan during vulnerable periods of neurodevelopment can alter the normal development and potential function of neurotransmission in the frontal cortex. Interestingly, the alterations identified in our study closely mimic the pathological markers associated with schizophrenia, which shows disturbances in synaptic proteins important for GABAergic, glutamatergic, and dopaminergic signaling in the frontal cortex. These findings provide important support for the impact of exposure to environmental chemicals during neurodevelopment and risk for neurological disease.

To cite this article: W. Michael Caudle. Vulnerability of synapses in the frontal cortex of mice developmentally exposed to an insecticide: Potential contribution to neuropsychiatric disease. Neurotransmitter 2015; 2: e514. doi: 10.14800/nt.514.
neurodevelopment have been found to underlie the pathology associated with several neurological diseases and disorders including, autism spectrum disorder, schizophrenia, depression, cognitive deficits, neurodegenerative disease, among many others. Recent advances in brain imaging and microscopy have substantially elaborated our understanding of the cellular and molecular targets that underlie the pathological dysfunction in many of these disorders. Accumulating evidence has delineated the neuronal synapse as a key target for dysfunction in many of these diseases [2,3]. Hallmark features of this pathology seem to focus on alterations in the expression and function of critical synaptic proteins involved in the establishment of pre and postsynaptic architecture as well as proteins involved in normal neurotransmission, thus contributing to shortcomings in circuit formation and function of the synapse. While several studies have implicated specific genes that may be involved in mediating these neurological deficits, more recent work has begun to appreciate the contribution of various exogenous factors as risk factors for many of these disorders.

To date, a variety of environmental factors have been shown to contribute to the etiopathology of many neurodevelopmental disorders, including exposure to alcohol and specific pharmaceuticals, including valproic acid, or the consequences of nutritional deficits during neurodevelopment. However, recent work has provided evidence that exposure to toxic environmental chemicals during critical periods of neurodevelopment can severely impair normal neurological function in children and contribute to disease [4,5]. A major class of environmental chemicals that has received significant attention is pesticides. These compounds are routinely applied to a variety of our fruits and vegetables, as well as other consumer crops in order to attenuate or abrogate loss or damage from pests. In general, pesticidal properties target specific neuronal proteins and functions that are present in the insect, including blockade of neuronal receptors, modification of select ion channels and transporters, and inhibition of mitochondrial respiration, resulting in the demise of the insect.

Recent work has focused on the neurological effects of exposure to the chlorinated insecticide, endosulfan. Like other insecticides, endosulfan specifically targets and inhibits GABA_A receptors, leading to hyperexcitation of the neuron [6]. These effects are particularly concerning as endosulfan tends to accumulate and persist at significant levels in the environment as well as in our bodies. Indeed, elevated levels of endosulfan have been routinely identified in fat and brain tissue, with significant concentrations found in cord blood and breast milk of the human population [7,8]. These findings suggest that children have the potential to be exposed to endosulfan, in utero, as well as postnatally during lactation. Increasing epidemiological evidence has identified exposure to chlorinated compounds during critical periods of neurodevelopment as a major risk factor for a host of neurobehavioral disorders [9-12]. In light of these findings, few studies have evaluated the contribution of developmental exposure to endosulfan and damage to key neurotransmitter circuits in specific regions of the brain, such as the frontal cortex that may be mediating many neurological deficits. While the clinical symptoms of many neurodevelopmental deficits are heterogeneous a unifying pathology comprised of alterations to the synapse of select neurotransmitter systems in the frontal cortex appears to underlie several of these disorders [2,3]. Thus, we hypothesized that developmental exposure to endosulfan would elicit alterations to select synaptic targets that serve the frontal cortex and are potentially damaged in several diseases. Specifically, we concentrated our study on assessing damage to synaptic proteins involved in functioning of the GABAergic, glutamatergic, and dopaminergic neurotransmitter circuits. These pathways were chosen given their extensive innervation and importance in several cognitive processes in the frontal cortex [13,14].

To address impact of neurodevelopmental exposure to endosulfan we applied complementary in vitro and in vivo experimental approaches aimed at characterizing the neuronal targets most vulnerable to endosulfan [15]. First, in order to identify specific aspects of neuronal morphology that may be affected by endosulfan we coupled primary cultured neurons isolated from the frontal cortex of neonatal mice with high content imaging. This approach allowed for the capability to evaluate discrete changes to cortical neurons, particularly related to loss of cortical neurons, growth of neuronal processes, and the development of synaptic connections, as these are critical aspects of neurodevelopment that may underlie neurobehavioral deficits. Exposure of frontal cortical cultures to low concentrations of endosulfan did not result in an appreciable reduction in the number of cortical neurons in culture. However, at the same concentrations, more subtle deficits were seen in the length of neuronal processes emanating from the neuronal cell body. As extension of these processes is critical in establishing connectivity between neurons, a reduction in the length of the neurites would suggest an encumbered ability to establish a functional neuronal circuitry. In addition, an even greater reduction in the number of synapsin-positive puncta was observed in the endosulfan treated cortical cultures. In our assay, synapsin-positive puncta were used as a proxy to identify and quantify the formation of synapses between neurons, thus providing an indicator of synaptogenesis. With a substantial reduction in synapsin-positive puncta in our cultures we were able to
conclude that neuronal mechanisms involved in the establishment of neuronal connections, including neurite outgrowth and synapse formation are particularly sensitive to endosulfan exposure. Thus, we leveraged these data to refine our subsequent in vivo studies, allowing focused investigation of alterations in specific synaptic proteins of neurotransmitter populations highly localized to the frontal cortex, particularly GABA, glutamate, and dopamine.

As many neurobehavioral disorders are believed to arise from impairments in neurodevelopment we applied an exposure paradigm that recapitulated developmental exposure to endosulfan in the human population. Given the persistence of endosulfan in the environment and human tissue we first exposed female mice to endosulfan in order to establish a general body burden of the compound. Mice were then bred with male mice and exposure to endosulfan continued throughout the duration of gestation as well as during the time period of lactation, again simulating an exposure scenario similar to that seen in humans. At 4 months of age male offspring were evaluated for alterations to a suite of synaptic markers involved in GABAergic, glutamatergic, and dopaminergic signaling in the frontal cortex. In male offspring significant alteration in GABAergic synapses was observed, with a substantial reduction in the expression of the vesicular GABA transporter (vGAT) as well as the plasma membrane GABA transporter (GAT1). In contrast, we also observed an elevation in the expression of the postsynaptic GABA$_{\alpha}$ 2$\alpha$ receptor subunit. The intimate interplay between presynaptic processes and postsynaptic targets serve to tightly regulate GABAergic signaling in the synapse and disruption could contribute to an imbalance in inhibitory tone in the frontal cortex.

In addition to being highly innervated by GABAergic neurons, the frontal cortex also has extensive connections with glutamatergic neurons as well as receiving extensive mesocortical dopaminergic projections. Similar to our findings in the GABAergic neurons, we found alterations to synaptic proteins in both the glutamatergic and dopaminergic circuits in the frontal cortex. A significant increase in expression of the vesicular glutamate transporter (vGlut), which resides in the presynaptic terminal of glutamatergic neurons and a concomitant reduction in expression of the postsynaptic glutamate receptor 2B (GluN 2B) was also observed in the frontal cortex of mice developmentally exposed to endosulfan. As with the GABAergic findings, these alterations suggest a general deficit in glutamatergic signaling in the frontal cortex. Indeed, it can be speculated that an increase in packaging of glutamate into synaptic vesicles due to an increase in vGlut expression could result in elevated levels of glutamate being released into the synapse during neurotransmission. As a potential feedback or compensatory mechanism aimed at reducing the activation of the postsynaptic neurons, GluN 2B receptors could be internalized from the plasma membrane, resulting in attenuated signaling through this circuit.

Finally, dopaminergic cell bodies that reside in the ventral tegmental area of the midbrain send a dense meshwork of dopaminergic projections to the frontal cortex. We were particularly interested in this circuit as prior work from our group had identified alterations in the nigrostriatal dopamine system in mice that had also been developmentally exposed to endosulfan [16]. Similar to our previous findings evaluation of dopaminergic proteins in the frontal cortex found substantial deficits in critical synaptic proteins involved in dopamine signaling in endosulfan exposed offspring. As with the GABAergic and glutamatergic circuits, alterations appeared to be preferential for neurotransmitter transporters and receptors, with reductions in the expression of the vesicular monoamine transporter 2 (VMAT2), the plasma membrane dopamine transporter (DAT), as well as tyrosine hydroxylase (TH), which functions as a key enzyme in the synthesis of dopamine. In contrast to these reductions, elevations of the postsynaptic dopamine receptor 2 (D2R) were seen. These alterations in select dopaminergic components that are imperative for normal dopamine signaling suggest significant disruption in the normal functioning of the dopamine system in the frontal cortex of animals developmentally exposed to endosulfan.

These data clearly demonstrate the deleterious repercussions of exposure to the organochlorine pesticide, endosulfan, during critical periods of neurodevelopment, with significant alterations to select synaptic proteins in the GABAergic, glutamatergic, and dopaminergic neurotransmitter circuits that innervate the frontal cortex. At face value, these findings provide the most extensive characterization of synaptic pathology following developmental exposure to endosulfan. However, when these data are evaluated from the context of relevance to neurological disease, a far more interesting picture begins to emerge. As discussed above, a diversity of neurological deficits have their root in pathology associated with the frontal cortex. However, as we completed our study we were struck by the similarities in our pathological findings in the frontal cortex and those seen in schizophrenia. Although schizophrenia is comprised of a constellation of abnormal behavioral features, disturbances in cognitive functions, including working memory and executive function are consistently observed and appear to be a prominent behavioral disturbance associated with the disease [17].

A wealth of evidence has demonstrated that alterations in GABA, glutamate, and dopamine neurotransmission in the
frontal cortex contribute to the cognitive disturbances seen in schizophrenia. Similar to our findings, several reports have identified disruption in several GABAergic proteins involved in mediating GABA signaling, including reduction in expression of GAT1 and an elevation of the GABA_\alpha$_{2\alpha}$ receptor subunit have been identified in postmortem samples from patients with schizophrenia and have been suggested to underlie the reduction in inhibitory input in the frontal cortex [18-20]. Cognitive deficits have also been attributed to a hypofunctioning of the NMDA receptors, resulting in a disturbance of glutamatergic signaling in the frontal cortex [21-23]. Our data also suggest a disruption of glutamatergic neurotransmission in the frontal cortex of animals exposed to endosulfan, which showed an elevation in the expression of vGlut and a contrasting reduction in the NMDA receptor, GluN 2B. These findings are interesting as a good deal of research has focused on the GluN 2B and have identified mutations in this gene to be a risk factor for schizophrenia [24]. Finally, it has been suggested that changes to various aspects of dopamine signaling, including dopamine storage, vesicular transport, dopamine release and reuptake could underlie many of the symptoms associated with alterations in executive function and memory in patients with schizophrenia. While genetic alterations in the VMAT2 gene have been identified as a risk factor for the cognitive deficits observed in schizophrenia, other studies have found a significant reduction in DAT in the frontal cortex of schizophrenic patients [25, 26]. Further work focused on assessing the integrity of the dopaminergic projections to the frontal cortex found a reduction in the dopaminergic innervation of layer 6 of the frontal cortex in postmortem samples taken from patients with schizophrenia [27]. These findings align very well with our data that demonstrated substantial reductions in several presynaptic proteins, including DAT, VMAT2, and TH that are highly involved in mediating normal dopamine signaling in the frontal cortex.

These findings provide a compelling argument that exposure to endosulfan during critical periods of neurodevelopment could be an environmental risk factor for the development of schizophrenia. The contribution of external factors in the etiology of schizophrenia is not new. Numerous reports have identified multiple exogenous insults, including paternal age, prenatal immune activation, and perinatal hypoxia, among others, as risk factors for the disease [28-30]. Combined with our understanding that neurodevelopment represents a uniquely vulnerable period of brain growth that is highly susceptible to disruption by exogenous mediators, these findings raise concern about children’s exposure to chemicals, such as pesticides. The importance of these findings are further reinforced as recent work has focused on the possibility that neurological diseases, such as schizophrenia may have their origins in damage to sensitive neurodevelopmental processes, particularly in the formation of GABAergic circuitry in the frontal cortex [31, 32]. Future work with our model will focus on further characterizing the molecular alterations to specific neurotransmitter systems in the frontal cortex and undertaking a critically needed behavioral assessment of these animals that will allow us to identify the behavioral correlates of our established neuropathology.

Acknowledgements

Funding for this project was generously supported by the National Institutes of Health, (R00ES017477 and P30ES019776).

References


19. Volk DW and Lewis DA. Impaired prefrontal inhibition in schizophrenia: relevance for cognitive dysfunction. Physiol Behav 2002; 77:501-505.

20. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 2005; 6:312-324.


22. Kantrowitz JT and Javitt DC. N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? Brain Res Bull 2010; 83:108-121.


