Genetics of Childhood Disorders: LV. Prenatal Drug Exposure

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The topic of the reviews in this series will be prenatal exposure to drugs such as cocaine and alcohol and their effects on neural ontogeny and associated neurocognitive/neurobehavioral development in offspring. Exposure to drugs prenatally, surely an important concern for practitioners, is part of the broader field of behavioral teratology or the study of perinatal developmental injury and of the factors—including drug and toxin exposure, parental disease or impairment, and birth accidents—that increase the risk of immediate or later physical injury or developmental impairment.

Some of the earliest questions in this field were about the effects of maternal malnutrition and of irradiation on fetal and infant neurodevelopmental outcome, and the most contemporary questions are about the impact of maternal stress and anxiety on long-term infant and child neurodevelopment. Questions regarding the impact of exposures to exogenous agents or events during specific developmental phases are implicit in nearly every consideration of a psychiatric disorder or the manifestations of that disorder across development. The field of behavioral teratology has grown far past a strictly behavioral emphasis to include functional neuroimaging, neurophysiology, neurochemistry, and neuropsychology. Indeed, interest in developmental injury from a psychological/behavioral point of view has facilitated progress in understanding the normal or expectable features of neural development and early perceptual, social-emotional, and cognitive processes.

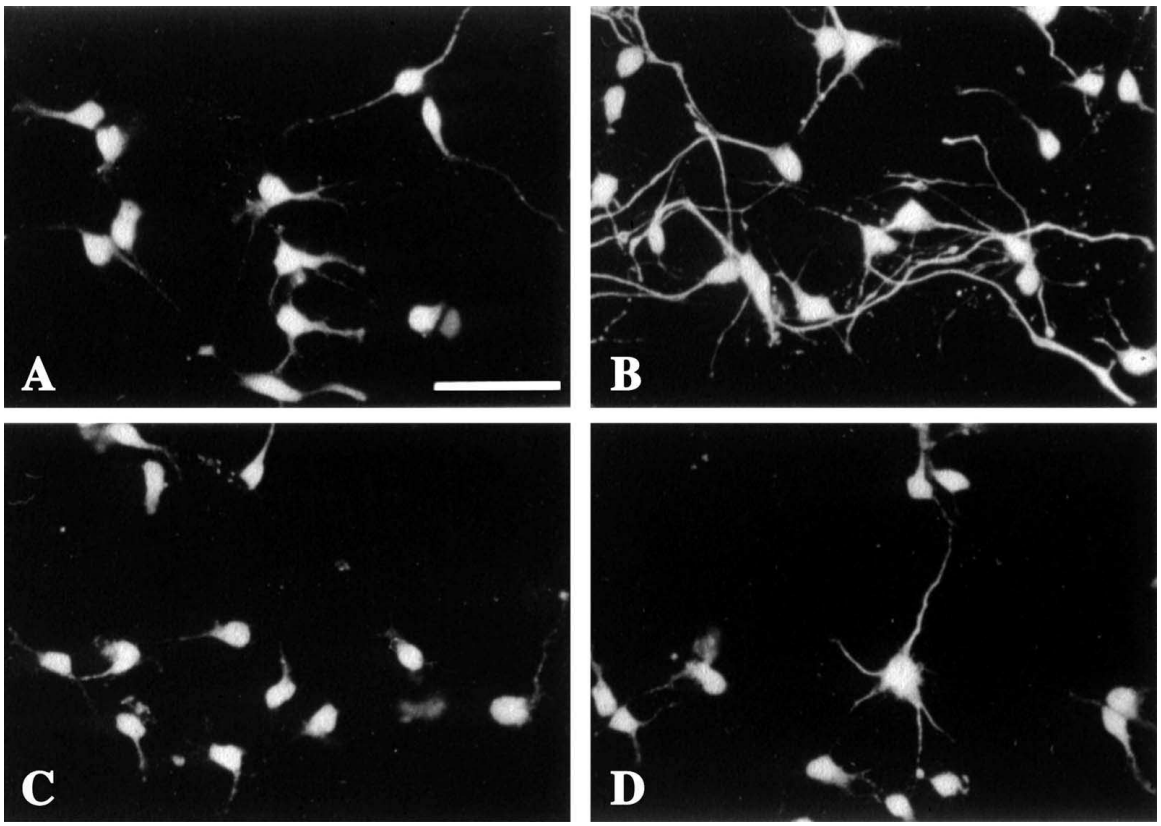

Fig. 1 MAP2-stained medial frontal cortical of 21-day-old rat embryos (A, B) (including anterior cingulate cortex) (ACC) and visual cortical (C, D) culture preparations from saline-exposed (A, C) and cocaine-exposed (B, D) embryos. ACC cocaine-exposed neurons (B) have longer neurites than ACC-saline (A), visual cortical-saline (C), and visual cortical-cocaine (D). From Jones LB, Stanwood GD, Reinoso BS, Washington RA, Wang H-Y, Friedman E, Levitt P (2000), In utero cocaine-induced dysfunction of dopamine D_1 receptor signaling and abnormal differentiation of cerebral cortical neurons. *J Neurosci* 20:4606–4614; copyright 2000 by the Society for Neuroscience.

Behavioral teratological questions about developmental injury may be approached from two broad perspectives. One perspective examines presumed causes, that is, by grouping subjects into those with and without the presumed cause of injury, such as exposure to a specific drug that produces mutations or other malformations (e.g., alcohol). Another perspective studies developmental injury through the target organ, system, or function of injury. Subjects are grouped by their functional impairment, and the programmatic focus is to understand the various mechanisms and routes to that particular functional impairment. These two orientations may lead to different research questions, findings, and interpretations because one focuses on outcome while the other emphasizes mechanism. The most productive approach to any investigation of developmental injury brings a combination of these two perspectives and a plurality of methods to the research questions.

The fundamental questions from behavioral teratology are the following: Does exposure to X during a specific phase of development cause A, B, C and/or D immediately or later in development? In the language of mechanism, does disruption in process X during a specific phase of development lead to disruptions in functions A, B, C, and/or D later in development? One agent may produce several different outcomes depending on dose, amount, or timing of exposure, and there may be different dose-response curves for the different outcomes. The shape of the dose-response curves may be different depending on the outcome of interest. That is, one outcome may reflect a linear relationship with exposure—more impairment with more exposure, while another outcome may reflect a threshold effect—only exposures above a certain amount or time are teratogenic.

Behavioral or neuropsychological changes usually occur at lower doses than are necessary to lead to abnormal growth or disruptions of other organ systems. This is why assurances regarding lack of association with birth defects or physical malformations are not sufficient to assuage concerns regarding effects on neurobehavioral development.

There are several classic examples of direct causative links between exposures and functional/physical outcomes. These include prenatal rubella exposure and its association with deafness and mental retardation or the classic example of prenatal thalidomide exposure and severe malformations of limb development. In these models, a specific exposure to a discrete toxin occurs and a clearly defined and easily identified outcome emerges.

However, many, if not most, of the more contemporary questions capturing the interest of clinical scholars are those that involve far more complex models of exposure, timing, and outcome assessment. These are not clearly direct causality models and hence are not easily approached with standard research designs. The exposures are neither specific nor discrete, the outcomes are not uniformly present even with documented exposure, and the severity or extent of the deformation or developmental abnormality is variable.

In these more complex models, interactions between the exposure agent or event and the environment are central. That is, does the environment in one way or another moderate the fetus's or child's risk of exposure, as well as vulnerability to the potentially toxic effects of exposure, and at the same time, determine other risk factors that may also mediate the severity of any exposure-related outcome? Both alcohol and cocaine are examples of these types of exposures. Each may have direct toxic effects on aspects of neural development. How these effects are expressed functionally across development depends on many other intervening and mediating conditions.

Several principles are key to evaluating the relation between any event or exposure and later developmental impairment. These include defining (1) the possible mechanisms of effect, (2) the specific teratogenic agent or event, (3) the timing of the exposure, (4) possible dose-response relations, (5) the outcomes most likely related to the mechanism of action of the exposure agent or event, (6) when exposure related outcomes are most likely to be apparent, and (7) those conditions that ameliorate or exacerbate any exposure-related functional outcomes. Studies of alcohol and cocaine exposure in both the preclinical and human models illustrate many of these principles. Any consideration of the association between an earlier exposure and a later outcome needs to consider these principles as essential criteria for establishing more than putative links.

Linking putative outcome to proposed mechanisms is perhaps the most important of these principles. While it may seem obvious that it is important to consider the possible mechanisms of teratogenic effects of any given agent, it is not always the case, particularly in studies of humans, that mechanisms of effect are specified or hypothesized, beyond the general expectation or assumption that, for example, psychoactive drugs administered during active CNS neurogenesis should be potentially teratogenic. This assumption not only ignores consideration of specificity of effect on particular CNS regions and functions but also does not permit a more hypothesis-driven consideration of the possible domains of outcome to study. Often possible mechanisms of action are defined, not through investigations of the specific agent, but rather through studies of other agents with similar mechanisms of action in the brain. For example, the potential effects of cocaine on developing monoaminergic systems in the fetal brain have been delineated through in vitro and in vivo studies of monoaminergic regulation of neurogenesis, neuronal migration, and synaptogenesis, as well as through direct study of disruptions in fetal brain structure–function relations with cocaine exposure.

Consider the following line of work regarding the impact of cocaine on signal transduction in the mesocortical dopaminergic system. This line of work illustrates the interplay between focusing on the teratological impact of a potentially toxic agent and using the possible impact of that agent to

inform models of normative development, as well as delineating hypothesized mechanisms of effect for that agent on behavior and neurocognitive capacities. Because one of the primary mechanisms of action of cocaine in the mature animal is to block presynaptic transporters of monoamines and thus presynaptic monoamine reuptake, it is reasonable to ask what might be the impact of increased synaptic amine concentration during the prenatal period? On the basis of the critical role monoamines play in neuronal migration, maturation, and synaptogenesis, what might be the expected structural and functional impact of disrupted monoamine metabolism during these different phases of cortical development? Normative data show that monoamine receptors are expressed early in corticogenesis. The dopamine D_1 receptor is distributed on the proliferating cells of the ventricular zone and on postmitotic cortical neurons. Thus it is reasonable to examine those regions of developing cortex rich in dopaminergic innervation, especially the anterior cingulate and prefrontal cortex.

Studies targeting the structural properties of the mesocortical dopaminergic system have shown that prenatal cocaine exposure in animals is associated with abnormally elongated dendrites in corticolimbic pyramidal neurons. Dendrites of pyramidal neurons in layers III and V of the anterior cingulate cortex are 30% to 50% longer in exposed animals compared with nonexposed, saline-treated animals. *Confocal analysis*, a microscopic technique that permits much greater resolution of tissue samples than was possible only a few years ago, shows that these dendrites course abnormally through the cortex. Rather than the expected straight distribution, the dendrites are found to migrate in and out of the plane of a section. Since the exposed animals show normal lamination and thickness of cortical layers in the anterior cingulate, the trajectory pattern of the dendrites suggests less controlled growth, with the extended dendritic projections undulating to fit within the limits of the cortical layers. These morphological changes persist into adulthood. Similar observations are made in the prefrontal cortex, another cortical region targeted by dense dopaminergic afferents. These changes in dendritic growth are not found in regions that receive sparse dopaminergic input, such as the primary sensory cortex.

These effects on dendritic growth appear rapidly. In vitro studies show that after only 2 weeks of exposure to cocaine, fetal neurons from the anterior cingulate plated in culture without further cocaine exposure show marked increased in dendritic growth, a finding suggesting that the mechanism of the cocaine-related effect on growth is through an alteration in growth-regulating mechanisms.

How might cocaine interact with dopaminergic receptors in the developing brain to produce these structural changes? Careful studies of receptor-ligand binding and in situ hybridization of D_1 and D_2 receptors show no difference between exposed and nonexposed animals; that is, cocaine exposure does not appear to alter either the binding affinity or number of dopaminergic receptors. Similarly, no differences are found in dopaminergic afferents to the anterior cingulate cortex; that is, the region is no more or less dopaminergically innervated in exposed and nonexposed animals. However, D_1 receptor signaling appears altered in cocaine-exposed animals. Prenatal cocaine exposure impairs coupling in the frontal and cingulate areas of the dopaminergic D_1 receptor system to $G\alpha_S$ protein, a functional decrease that persists into adulthood. As we have discussed before in these columns, the stimulation of dopamine receptors leads to an association with different G proteins. $G\alpha_S$ leads to activation of adenyl cyclase to increase the second messenger cAMP. This in turn activates the protein kinase A cascade to phosphorylate different downstream substrate molecules. Coupling between D_2 and $G\alpha_i$ protein has the opposite effect and, in fact, remains normal after prenatal cocaine exposure. The uncoupling of D_1 to $G\alpha_S$ protein may be secondary to increased phosphorylation of the receptor that in turn renders the receptor less able to bind to the G protein complex.

What is the mechanism by which reduced coupling between D_1 and $G\alpha_S$ might result in the structural dendritic changes observed in exposed animals? Here is the important link to models of normal neuronal growth and to how monoamines regulate neuronal and glial differentiation. In vitro, dopamine modifies axonal and dendritic outgrowth through coupling to second-messenger systems. It appears that D_1 receptor activation decreases axonal and dendritic outgrowth in vitro whereas D_2 activation stimulates axon growth. Thus, in those regions in which D_1 receptors are functionally decoupled from their second messenger system, we might expect to see the increased and unregulated dendritic growth described in the anterior cingulate and prefrontal cortex of cocaine-exposed animals secondary to unopposed D_2 stimulation of growth. Taking these observations one step further, we might also ask whether there are additional mechanisms by which this alteration in receptor-second messenger coupling in the D_1 system might affect those cellular processes regulating neuronal growth? Again, this illustrates the interplay between questions of effect (e.g., what happens with the potentially teratogenic exposure?) and questions of mechanism (e.g., how does disruption in process X affect other maturational processes?).

In recent work in cocaine-exposed animals, elevated levels of mitogen-activated protein kinases (MAPKs) have been described in those regions in which altered dendritic morphology is also noted. MAPKs are highly expressed in brain and are central signaling molecules that translate extracellular signals to the cell nucleus and thus regulate cell proliferation and differentiation. Dopamine receptors differentially regulate members of the MAPK family, and D_1 receptor stimulation activates p38 MAPK, which in turn stimulates dendritic outgrowth. With receptor hyperphosphorylation cited above, the D_1 receptor is switched over from G protein coupling to the activation of p38 MAPK. Thus one possible

mechanism for the impact of prenatal cocaine exposure on dendritic morphology may be through the elevation of D_1-regulated p38 MAPK secondary to the decoupling of the D_1-$G\alpha_S$ system, in addition to the unopposed stimulatory effect of the D_2 system.

What might be the functional effects of this decoupled D_1-$G\alpha_S$ system? Several lines of work have addressed this question. In a mouse line with a targeted deletion of the D_1 receptor, animals show reduced responsivity to cocaine, further suggesting the relation between cocaine and the D_1 system. Second, in rodents, cocaine or stimulant administration in adulthood elicits a number of characteristic responses including head bobbing, increased sniffing, and activity, behaviors mediated in large part through the D_1 system. Selective D_1 antagonists abolish this characteristic behavioral response to cocaine as does prenatal cocaine exposure, presumably at least in part through the decoupling effect on the D_1-$G\alpha_S$ system. Third, the anterior cingulate cortex plays a central role in processes of learning and memory and is especially crucial to situations that demand preferential attention to less salient, but relevant, stimuli when more salient, but not necessarily relevant, stimuli occur in the same context. Animals exposed to cocaine prenatally demonstrate difficulty acquiring through conditioning the ability to make these responses, and these attentional deficits persist into adulthood. Findings of this nature parallel reports from studies of prenatally cocaine-exposed preschool and school-aged children, suggesting deficits in selective attention and in information processing.

This particular model of the effect of prenatal cocaine model indicates that it is possible to obtain very selective, targeted effects even with globally administered drugs if those drugs are experienced during specific phases of neural development. The findings of prenatal cocaine studies also suggest that monoamines in certain regions of the brain are critical in the modulation of specific morphogenetic events. Thus the prenatal cocaine exposure model informs an understanding of normal processes of neural ontogeny as well as illustrates the importance of grounding questions regarding the impact of prenatal toxins and other potentially adverse events in models of hypothesized mechanisms of effect.

WEB SITES OF INTEREST

http://www.eurekalert.org/pub_releases/2002-04/cwru-a2y041602.php
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