

Striatal Morphological and Functional Alterations Induced by Prenatal Alcohol Exposure

Yao-Ying Ma

Affiliation:

Department of Pharmacology and Toxicology
Indiana University School of Medicine
635 Barnhill Drive, MS A422
Indianapolis, IN 46202

Email:ym9@iu.edu

Phone: (317) 274-1536

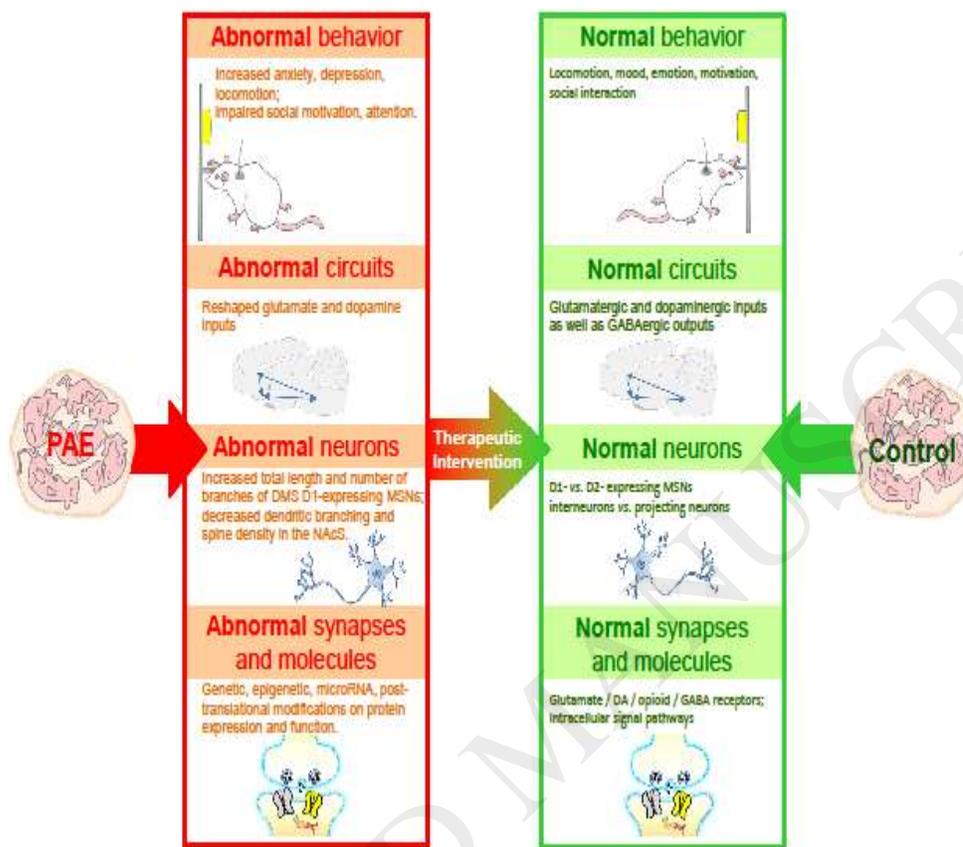
Fax: (317) 274-7714

Graphical abstract

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Abstract

Prenatal alcohol exposure (PAE) is an insidious yet preventable cause of developmental disability. The prenatal stage is a critical period for brain development with the concurrence of high vulnerability to the acute and prolonged effects of PAE. There is substantial evidence from both human observations and laboratory experiments that PAE is a common risk factor that predisposes to an array of postnatal mental disorders, including emotional, cognitive, and motor deficits. Although it is well accepted that PAE causes substantial morbidity, available treatments are limited. One reason is the lack of sufficient understanding about the neuroalterations induced by PAE, and how these changes contribute to PAE-induced mental disorders. Among a number of brain structures that have been explored extensively in PAE, the striatum has attracted great attention in the last 20 years in the field of PAE neurobiology. Interestingly, in animal models, the striatum has been considered as a pivotal switch of brain dysfunction induced by PAE, such as addiction, anxiety, depression, and neurodegeneration. In this review, we focus on recent advances in the understanding of morphological and functional changes in brain regions related to alterations after PAE, in particular the striatum. Because this region is central for behavior, emotion and cognition, there is an urgent need for more studies to uncover the PAE-induced alterations at the circuit, neuronal, synaptic and molecular levels, which will not only improve our

understanding of the neuroplasticity induced by PAE, but also provide novel biological targets to treat PAE-related mental disorders with translational significance.

Key Words:

Prenatal alcohol exposure; striatum; accumbens; neuroplasticity; medium-sized spiny neurons.

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Introduction

Prenatal alcohol exposure (PAE) is an insidious cause of developmental disability. Despite considerable and widespread knowledge that PAE can lead to devastating effects on the developing fetus, alcohol consumption by pregnant women remains strikingly prevalent. The estimated incidence of PAE-induced developmental disability or mental disease is 10 per 1000 live births (1). The prevalence of maternal alcohol consumption during pregnancy has been reported to be as high as 10%-16.3% (1), making the mechanistic study of these phenomena extremely important.

The prenatal stage, including all three trimesters, is a critical period with the concurrence of high vulnerability to the acute and prolonged effects of PAE and a key stage for brain development (2). PAE can have detrimental effects on brain development and behavior of the offspring at different developmental stages. The most critical birth defect associated with alcohol consumption is fetal alcohol syndrome (FAS), a common diagnosis in children with PAE history and displaying birth defects and a wide range of emotional, cognitive, and motor deficits. FAS prevalence in the US and some Western European countries is estimated at 2–5% of school children (3). However, many children who were prenatally exposed to alcohol do not present the morphological and functional brain changes characteristically seen in FAS, yet they may still exhibit many of the mental dysfunctions associated with PAE at a later developmental stage (4). PAE is a common risk factor that predisposes to an array of late-life mental disorders, including alcohol abuse, drug addiction, anxiety, depression and neurodegenerative diseases.

Although it is widely accepted that the behavioral, cognitive and emotional alterations induced by PAE cause substantial morbidity, available treatments are limited. One reason is the lack of sufficient understanding about the neuroadaptations induced by PAE, and how these changes contribute to PAE-induced mental disorders. Among a number of brain structures that have been explored extensively in PAE, including the cortex, basal ganglia and hippocampus, the striatum occupies a central position. Interestingly, in animal models, the striatum has been considered as a pivotal switch of brain dysfunction induced by PAE. In this review, we focus on recent advances in the understanding of morphological and functional changes in brain regions related to adaptations after PAE, in particular the striatum.

The striatum and its role in behavior, emotion and cognition: The striatum is implicated in multiple brain functions, such as locomotion, attention, motivation, habituation, mood, emotion, alcohol abuse and drug addiction. It is a complex, multi-component structure possessing a ventromedial-to-dorsolateral gradient structurally and functionally (5). It consists of at least 4 subregions including the dorsolateral striatum (DLS), dorsomedial striatum (DMS), nucleus accumbens (NAc) shell (NAcS), NAc core (NAcC). The striatum is a convergent unit collecting glutamatergic and dopamine (DA)ergic inputs necessary to generate adaptive commands in basal ganglia output regions. Heterogeneous afferents innervate striatal neurons, primarily ($\geq 90\%$) composed of medium-sized spiny neurons (MSNs). Excitatory glutamatergic inputs, originating from the cerebral cortex, thalamus, hippocampus and amygdala, and modulatory dopaminergic inputs, originating from the substantia nigra pars compacta and ventral tegmental area, directly converge onto the dendritic spines of the same MSNs. In turn, the striatal MSNs projects back to the frontal lobe of the cortex via direct and indirect pathways (6). In addition to the double regulation of MSNs by glutamatergic and DAergic inputs, the Mu, Delta and Kappa opioid receptors (MORs, DORs, and KORs), highly enriched in the striatum, also affect function by directly modifying the MSNs or modulating glutamatergic and dopaminergic inputs. The MOR, generally observed in both postsynaptic and presynaptic compartments (7), has been considered as the primary marker to distinguish the patch compartment (10–20% of the striatal volume, high MORs) and surrounding matrix ($>80\%$ of the striatal volume, low MORs) (8, 9). DORs appear to

be uniformly expressed in striatum. MORs, together with DORs, regulate MSN excitability and presynaptic release of DA and glutamate (10). KORs, which are predominantly distributed in the presynaptic terminals of the striatum, control synaptic DA concentration by influencing both the release and uptake of DA (11), which selectively modulates multiple glutamatergic inputs onto MSNs (12). Relatively few investigations of the striatal interneurons, either cholinergic or GABAergic, have been made in subjects with a PAE history.

Human and non-human primate studies on PAE: The consumption of alcohol during pregnancy can affect the brain and the postnatal behaviors in various ways. Epidemiological and pre-clinical studies have demonstrated that PAE is a key developmental factor that is associated with increased rates of illicit-substance use during adolescence and early adulthood (1, 13-15). Volumetric analysis of children diagnosed with FAS revealed an overall 13% smaller brain size compared to the brains of an age-and gender-balanced group of controls. These results confirmed those previously published in both laboratory animals and human autopsy studies. Compared with typical developing peers, brain volume decrements have been reported in human subjects with heavy PAE. In the PAE group, volume of the caudate nuclei was the most consistent predictor of neuropsychological performance, after controlling for potentially confounding variables including total brain volume, IQ, and age.

Between two measures of brain structure; volume and asymmetry, it was reported that caudate asymmetry (calculated by subtracting the caudate volume on the left from the right, divided by the total, i.e., $(V_R - V_L) / (V_R + V_L)$) is a more reliable neurobiological marker of mild to moderate PAE. Willford et al. (2010) reported that, although no significant effects of PAE on the volumes of either the left, or the right caudate striatum occur, young adults with PAE histories showed a more negative caudate asymmetry with larger left caudate striatum. This is consistent with the idea proposed by Sowell et al. (16) that regional asymmetries are a sensitive measure of changes in the structure and organization of the brain. Functional imaging studies also demonstrated significant changes in the striatum of subjects with PAE history. Heavy PAE (an average of ≥ 14 drinks per week or ≥ 4 alcoholic drinks per occasion at least once per week during gestation) results in an increased interhemispheric connectivity between the striatum at the infant stage (17) and a greater blood oxygen level-dependent (BOLD) response in striatum relative to the control group, especially as task difficulty increased, at the adolescent stage (18).

It is worth mentioning that nonhuman primate models of PAE been established (19-21). For example, moderate PAE models in rhesus monkeys can be generated by early exposure to 0.6 g/kg alcohol solution on GD 0 - GD 50; middle-to-late exposure to 0.6 g/kg alcohol solution on GD 50-GD 135; or continuous-exposure to 0.6 g/kg alcohol solution on GD 0 - GD135 (22). The application of primate models benefits studying complex behaviors and validating basic science findings in a system more like the human.

Rodent models of PAE: Besides the data from human cases and non-human primates, laboratory data have been widely collected from rodents. Modeling PAE in rodents has provided more mechanistic data essential for understanding the deleterious consequences of PAE at the morphological and functional levels. There are a few key factors to be considered when evaluating the data from different protocols of PAE.

Dose: The dose-dependent effects of PAE have been supported by substantial evidence. Although widely used, terms associated with consumption of alcohol--such as "light," "moderate," and "heavy"--are unstandardized (23). More often for rats, < 2 g/kg per day is considered as low dose, 2-3 g/kg as moderate dose and 4 g/kg or beyond as high dose. Higher doses are used for mice. For example, usually ~ 2.5 g/kg per day is considered as low dose, ~ 3.0 g/kg as moderate dose and 5 g/kg or beyond as high dose (24, 25). The dose of alcohol exposure is positively

related to the peak blood alcohol content (BAC) which measures the amount of alcohol per unit of blood (usually mg/dl) and this measure is typically taken within 2-4 hours after exposure.

Timing: First, PAE can be induced by an acute exposure on a single day of gestation, such as gestation day (GD) 8 (19, 26-28) or GD12 (29, 30). It has been demonstrated that the one-day exposure during pregnancy leads to high sensitivity to PAE insults. For example, rats acutely exposed to ethanol on GD12 show decreased social motivation at postnatal day (PD) 42 (29). Male offspring of animals exposed on GD12 exhibit deficits in play fighting (following, chasing, nape attacks, or pinning), contact behavior (grooming or crawling over/under the partner), and social investigation during adolescence and adulthood. Furthermore, GD-12 ethanol-exposed males and females showed evidence of social avoidance at PD 42 and PD 75 (30). Second, PAE may cover a broad period during pregnancy, such as GD 0 – GD 20 (31), GD 5 – GD 20 (32), GD 8 – GD 20 (33), GD 17-GD 20 (34, 35) or GD 19-GD 20 (36, 37). Furthermore, the three-trimester model of PAE in rodents entails alcohol exposure during the prenatal and early PDs (usually PD 2–10) [6-8], which is also called perinatal alcohol exposure and equivalent to all three trimesters in the human [9, 10]. Lastly, there are a few studies modeling PAE by alcohol exposure starting 30 days before mating, and throughout their gestational period in rats (38).

Route of administration: Administration of alcohol most commonly used in laboratory research includes but is not limited to drinking alcohol containing liquid diet, oral / intragastric intubation, vapor inhalation (reviewed by Abate et al., 2014 (39)). Although passive alcohol administration (such as intubation) is more often used, voluntary alcohol consumption (40, 41) may provide enhanced validity due to its closely mimicking human alcohol intake.

Postnatal behavioral consequences of PAE: Animals with PAE history are impaired as shown in striatum-dependent place and cue learning strategies measured by the Morris water maze task, which can be ameliorated by postnatal environmental enrichment (42). In animal models, PAE results in various neurobehavioral maladaptations in the striatum that are thought to underlie greater risk for addictions, including: elevated anxiety/depression-like behavior (43, 44), enhanced locomotor activity (45, 46), impaired social motivation, i.e., transformation of social preference into social avoidance (30, 47), and augmented hypothalamic-pituitary-adrenal axis activity (48). Laboratory animal models of PAE have also indicated a facilitatory effect of postnatal ethanol consumption at PD14 and PD15, particularly in females (39) and during the adolescent stage, which has been further demonstrated as a persistent effect through adolescence (49). Furthermore, PAE mice or rats showed attention deficits and impulsivity (50).

Morphological changes in the striatum after PAE: In contrast to human data, in C57BL/6J mice the shape of the striatum but not its volume was changed in adolescent C57BL/6J mice with PAE history (51). PAE-induced morphological adaptations showed a sub-regional and subcellular specificity. First, very recent laboratory animal studies showed that PAE increased total length and number of branches of DMS D1-expressing MSNs in adult offspring. These findings suggest that PAE triggers a long-term functional and structural plasticity in DMS D1-MSNs, potentially contributing to hyperactivity in both juvenile and adult offspring (40). Second, relative to saccharin controls, robust reductions in dendritic branching and length, but not spine density, were observed in NAcS of PAE rats. No significant PAE effects were found in NAcC, DLS and DMS (52). These findings suggest that PAE can have profound effects on brain regions related to reward processing and provide possible clues relevant to understanding increased self-administration of drugs of abuse in animals exposed to ethanol during brain development. Third, PAE during the 3rd trimester did not affect dendritic morphology or cell number in the NAc, although dendritic morphology in mPFC was significantly impacted (53).

The morphological changes of the MSNs can be evaluated not only by somatic size and dendritic complexity as shown above, but also through dendritic spine density and spine morphology. The

dendritic spines can be divided into two large categories: the filopodia-like protrusion is operationally defined as a protrusion without a clear head or with a head with a diameter <2 times the diameter of its neck; the mushroom-like protrusion is operationally defined as a headed protrusion with a diameter >2 times of its neck (54). The filopodia-like protrusion is proposed to indicate the immature or silent synapses and the mushroom-like protrusion is proposed to indicate the mature synaptic contact. Our unpublished data showed a decreased number of total spines but an increased ratio of mushroom-like / filopodia-like protrusions in the NAcS of rats with PAE history, indicating enhanced process of pruning and maturation in the NAcS induced by PAE.

Electrophysiological alterations in striatal synaptic transmission: Synaptic plasticity in cortical afferents to the DLS is involved in the pathogenesis of hyperlocomotion. High-frequency stimulation (HFS)-induced long-term potentiation (LTP) was weaker in PAE rats than that in control rats at PD 15. The same protocol of HFS induced long-term depression (LTD) in control group but still LTP in PAE group at PD 30 or PD 40. PAE rats showed a D1R-mediated potentiation of basal synaptic transmission through increasing presynaptic glutamate release (55). Very recently, Cheng and colleagues reported that PAE enhances AMPA receptor-mediated excitatory synaptic transmission in DMS D1-MSNs in adult offspring (40). Furthermore, our unpublished data showed an accelerated synaptic maturation/pruning within 4 weeks after birth in rats treated with ethanol prenatally and this acceleration was observed in the NAcS but not NAcC. Our data also indicated that this accelerated synaptic maturation/pruning in the shell may be attributable to increased proportion of Ca²⁺-permeable AMPA receptors which are GluA2 subunit-lacking. Finally, potential modifications of striatal glutamatergic and GABAergic neurotransmission were detected in PAE treated animals, which showed a decrease of the K⁺-induced release of glutamate in the control group (56).

Receptor alterations after PAE: Ethanol-induced changes in opioidergic transmission have been extensively studied in adult organisms. Similar levels of δ and κ opioid receptor mRNA were observed in the NAc as well as VTA and infralimbic cortex, but μ receptor mRNA in the VTA was significantly increased by PAE (49). 4-, 8-, and 12-day old PAE rats were detected with (a) upregulated mRNA of the three opioid receptors in the NAc, indicative of escalating ontogenetic expression of opioid-related genes; and (b) significantly reduced multiple opioid peptides, by which prenatal exposure can affect future responsiveness towards ethanol or drugs of abuse (34). Met-enkephalin expression in the NAc and the dorsal stratum was increased as a consequence of PAE, indicating a role of mesocorticolimbic enkephalins in ethanol reinforcement in offspring (39). Met-enkephalin levels in the NAc of female PAE rats were significantly higher than those in the control females (57).

PAE can reshape the DA system by multiple mechanisms, including changing the receptor-binding rate, modifying DA synthesis, and regulating the expression of DA receptor transporters, which has been supported by the studies below. First, PAE rats exposed to ethanol during GD (-30) – GD +20 (i.e., ethanol exposure starting 30 days before mating, and throughout their gestational period), showed a decrease in the striatal dopaminergic (D2: -30%; D1: -52%) and muscarinic (+42%) binding rate on PD 21 (38). Second, in adult rhesus monkeys with a history of PAE, the interaction between PAE and gender was observed in prefrontal cortex at a statistically higher level and in the NAc with a trend. It showed that PAE led to increased D1 receptor binding in male monkeys. This may help explain gender differences in the prevalence of neurodevelopmental disorders induced by PAE (58). Third, Schneider and colleagues (2005) found that the D2R binding/DA synthesis ratio was related to neonatal neurobehavioral measures (e.g., orientation, motor maturity, motor activity, and state control, etc.) in control monkeys, but these relationships were disrupted in the PAE monkeys (59). PAE monkeys from three different gestational alcohol exposures (early PAE, middle to late PAE, and continuous PAE) showed

disrupted striatal DA system function in slightly different ways. Early PAE, a period that is comparable to the first trimester in humans, or continuous PAE, reduced the ratio of D2R binding / DA-synthesis. Middle to late PAE, surprisingly, by itself increased the ratio of D2R binding / DA-synthesis. Generally speaking, continuously exposed monkeys showed the largest effect, suggesting that the sooner women stop drinking, the better it is for the fetus (59). Fourth, beside studies focusing on the DA receptor binding rate, the PAE group showed increased expression of DA and norepinephrine transporters (50).

Changes in other regulatory molecules after PAE: Long-term neuronal adaptations have to engage in modifying the expression of proteins. Besides the traditional modifications of protein expression, such as the transcriptional, translational and post-translational modifications on the cDNA, mRNA and the synthesized proteins, respectively, protein expression can also be modified by microRNAs (miRNAs), a group of small non-coding RNA molecules that function in RNA silencing and post-transcriptional regulation of gene expression (60). Several miRNA changes in the NAC caused by PAE on GD 12 were reversed by social enrichment (i.e., co-housed with 2-3 novel rats from untreated dams) after weaning, including mir-204, mir-299a, miR-384-5p, miR-222-3p, miR-301b-3p, and mir-6239 (29). Another way of non-traditional modifications on protein expression is the epigenetic modification, for example, MeCP2 coded by MECPT2 gene regulates gene expression by modifying chromatin (61). The PAE group showed decreased expression of MeCP2 protein in the striatum (50). A number of novel epigenetic mechanisms are suggested, particularly for social enrichment, to reverse the effects of PAE through extensive influences on gene expression. The expression of brain-derived neurotrophic factor (BDNF), which can be regulated by either miRNA or epigenetics, was decreased in PAE rats, which also can be ameliorated by postnatal environmental enrichment (42).

Future studies

Environmental enrichment or social enrichment has long been known to improve motor and cognitive function levels, causes noticeable neurochemical and morphological alterations in the brain. Housing rats that had been prenatally exposed to ethanol with peers that had regular prenatal and postnatal experiences, could ameliorate the social deficits seen in the PAE rats (30). This is a good example to explore potential treatments by rescuing the PAE-induced effects in rodents. There is an urgent need to perform translational research in the PAE model. However, the success of translational research depends on a solid knowledge of the mechanisms. The striatum circuit-specific (glutamatergic, GABAergic and dopaminergic input as well as GABAergic outputs), neuron-specific (D1- vs. D2- expressing MSNs, interneurons vs. projecting neurons (MSNs)), synaptic-specific (mediated by NMDA receptors, AMPA receptors, DA1/DA2 receptors, opioid receptors, GABA receptors, etc.) investigations are still at the early stage and more experiments need to be done to have a more complete and detailed picture of the PAE-induced changes in striatum.

Conclusions

The consumption of alcohol during pregnancy affects the development of the unborn offspring and leads to prolonged consequences in postnatal cognition, locomotion and mental status. The striatum, as an integral part of the basal ganglia and playing pivotal roles in behavior, motivation, emotion and mood, has been demonstrated to be highly sensitive to the effects of PAE by both human observations and animal experiments. Here we reviewed the research of striatum in PAE subjects in the past 20 years. Although the progress covered multiple aspects of striatum, more in-depth studies are needed to uncover the PAE-induced adaptations in the striatum at the circuit, neuronal, synaptic and molecular levels, which will not only improve our understanding of the neuroplasticity induced by PAE, but also provide novel biological targets to treat PAE-related mental disorders with translational significance.

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