Disruptions to hippocampal adult neurogenesis in rodent models of fetal alcohol spectrum disorders

Karen E. Boschen and Anna Y. Klintsova

Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, USA

ABSTRACT
Exposure of the embryo and fetus to alcohol can lead to abnormal physical, neuroanatomical, and behavioral development, collectively known as Fetal Alcohol Spectrum Disorders (FASDs). This mini-review focuses on the negative impact of prenatal alcohol exposure on hippocampal adult neurogenesis, an important process by which the brain adds new neurons throughout the lifespan, and hippocampal dendritic complexity through the discussion of various mammalian models of FASDs. Alcohol-induced aberrations in the outgrowth, phenotype, and stability of dendrites of neurons in the hippocampus and the prefrontal cortex will also be discussed. Timing of alcohol exposure during development (first trimester vs. third trimester-equivalent) can determine whether cell proliferation or long-term cell survival is impaired. Our work demonstrating that third trimester-equivalent exposure has a more significant impact on cell survival and dendritic morphology than rate of cell proliferation. Understanding the impact of prenatal ethanol exposure on adult neurogenesis is important as altered rates of new cell generation or successful integration of adult-born neurons could contribute to many of the hippocampal-associated deficits in memory and cognitive function observed in patients with FASDs. In addition, this commentary discusses evidence in support of aerobic exercise and environmental complexity (“enrichment”) as potential therapeutic strategies for alcohol-related deficits.

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Introduction
Adult neurogenesis occurs in 2 specific brain regions across the lifespan: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus. The cells generated in these 2 regions have distinct fates. Progenitor cells originating in the SVZ migrate via the rostral migratory stream to the olfactory bulb where they mature into interneurons, while the majority of adult-born cells in the SGZ differentiate into dentate gyrus granule cells and migrate into the granule cell layer. There is a growing body of evidence that hippocampal adult neurogenesis is important for memory formation, cognition, and behaviors associated with the hippocampus (reviewed in1). Newly generated granule cells receive excitatory synaptic connections from the entorhinal cortex and are indistinguishable from the preexisting granule cell population within 6–8 weeks since their birth. Adult neurogenesis is separated into stages of cell generation and maturation (as depicted in Fig. 1), including initial cell proliferation (1A), differentiation into either a neuronal or glial fate (1B), migration of the new neurons into the granule cell layer, extension of neurites and initiation of synaptic contacts (1C-D), and long-term survival (dependent on successful synaptic integration). Various specific endogenous proteins can be used to label cells at different stages of maturation (Fig. 1). Stem cells and neural progenitors are commonly labeled for the nuclear proteins nestin, Ki-67, GFAP, or Sox2, though the specific populations labeled with the proteins differ (e.g. Ki-67 labels actively dividing progenitor cells only, not the entire progenitor pool). The number of immature neurons can be measured using the endogenous markers doublecortin (DCX), polysialylated-neural cell adhesion molecule (PSA-NCAM), or NeuroD, which label neuronal progenitors and immature neurons ranging from a few days to 2 weeks old. DCX is a cytoskeletal protein, while PSA-NCAM is expressed in the membrane and NeuroD is restricted to the nucleus. In mice, calretinin is also expressed in immature neurons; however calretinin is not reliably

CONTACT Anna Y. Klintsova klintsov@psych.udel.edu 225 Wolf Hall, Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, 19716, USA.

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expressed in cell types besides inhibitory interneurons in other animal models, making it a useful marker both more specific and limited. Mature granule cells can be labeled with NeuN or the calcium-binding protein calbindin. Exogenously administered markers such as green fluorescent proteins (delivered as fluorescent reporter genes by retroviral injection) or thymidine analog bromodeoxyuridine (BrdU) can also be used to determine cell age, as these compounds are incorporated into the cell DNA during active division.

**Prenatal alcohol exposure and proliferation of adult-born hippocampal granule cells**

Various environmental influences can affect the process of adult neurogenesis, including its disruption by stress or drug exposure or enhancement by exercise or exposure to hippocampal-associated learning paradigms.\(^3\)\(^4\)\(^5\)\(^6\) Exposure of the fetus to alcohol during the prenatal period can have a devastating effect on the offspring, including craniofacial dysmorphologies and negative behavioral and cognitive outcomes. The range of deficits caused by prenatal alcohol exposure are collectively known as Fetal Alcohol Spectrum Disorders (FASD) and are estimated to affect up to 5% of live births each year in the United States.\(^7\) Among the cognitive impairments often observed in individuals with FASDs are spatial memory deficits, such as difficulty performing on a virtual water maze,\(^8\) suggesting individuals with prenatal alcohol exposure have some degree of hippocampal damage.

Damage to the hippocampus following in utero alcohol exposure has been borne out through rodent models of FASD. Behavioral performance on tasks relying on intact hippocampal function and adult neurogenesis, such as the Morris Water Maze or contextual fear conditioning, are often impaired in rodents exposed to alcohol either prenatally or during the early postnatal period (modeling the third trimester in human pregnancy). Most relevant to the current review, these models demonstrate that alcohol exposure during initial brain development can reduce the health of neural progenitors in the adult hippocampus and the ability of adult born granule cells to successfully mature and integrate into the hippocampal trisynaptic circuit. While the literature regarding this phenomenon is mixed, factors such as timing of alcohol exposure during development, peak blood alcohol concentration reached, and how adult neurogenesis is measured likely accounts for much of this variability.

Rodent models of FASD can model alcohol exposure during specific developmental events (e.g., gastrulation or neurulation), target specific trimesters of pregnancy, or administer alcohol throughout the duration of gestation. First and second trimester-equivalent exposure is modeled by administering alcohol to pregnant dams through gavage, injection, or vapor. The third trimester-equivalent occurs postnataally in rodents, with a steep increase in brain growth occurring during the first 2 postnatal weeks. Thus, to target developmental processes occurring during the third trimester, alcohol is delivered directly to the pups following birth, either through intragastric intubation, injection, or vapor. Alcohol most profoundly affects developmental processes occurring during

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*Figure 1. Schematic of the maturation stages of hippocampal adult neurogenesis and associated cellular markers. A) Proliferating cell pool located in the subgranular zone (SGZ) labeled by markers including Ki-67, Sox2, Nestin, and GFAP. B) Postmitotic neuronal progenitors begin to migrate out of the SGZ into the granule cell layer (GCL). These cells express doublecortin (DCX) and have very short neurite extensions. C)–D) Immature neurons continue to express DCX and in the later stages begin to express NeuN. E) After 4–6 weeks, adult-born neurons make functional, excitatory synapses with projections from entorhinal cortex pyramidal cells (highlighted in the red box). Mature granule cells express both NeuN and calcium-binding protein calbindin. By 8 weeks following proliferation, these new neurons are indistinguishable from the older granule cell population.*

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exposure or subsequent stages that rely on successful completion of events that are disrupted by alcohol exposure. While all stages of adult neurogenesis have been found to be affected in models of FASD, current evidence suggests alcohol exposure that targets early gestational time points has a more consistent and severe impact on hippocampal cell proliferation compared with cell survival. Redila and colleagues\textsuperscript{9} reported that the size of the progenitor pool and the number of proliferating neurons was reduced following prenatal alcohol exposure (equivalent to the first 2 trimesters) in a rat model of FASD. A single binge exposure on PD7 was shown to disrupt hippocampal cell proliferation in adult mice.\textsuperscript{10} Recently, prenatal alcohol exposure was found to disrupt olfactory bulb and hilar cell proliferation in infant and juvenile vervet monkeys.\textsuperscript{11} Alcohol could alter cell proliferation through various pathways. Ieraci and Herrera\textsuperscript{10} reported increases in caspase-3 co-localization with progenitor cell markers 12 hours following the alcohol exposure, suggesting apoptosis within the neonatal hippocampal progenitor pool as a potential source of disrupted neurogenesis in the adult brain. In addition, studies have suggested elongation of the cell cycle as a contributing factor in decreased cell proliferation\textsuperscript{12,13} in models of FASDs. Interestingly, adolescent alcohol exposure was found to target the S-phase of the cell cycle in neural progenitors in the rat dentate gyrus, decreasing the time spent in this stage and leading to a small, but significant, increase in cell proliferation.\textsuperscript{14} Alcohol-induced changes to neurotransmitter and growth factor expression necessary for normal stem cell regulation are also likely contributing factors.

Deficits in neuronal maturation processes and survival have been some of the most consistently reported alcohol-related effects on adult neurogenesis, particularly in postnatal rodent models of FASDs.\textsuperscript{10,15-18} A commonly used postnatal model of alcohol exposure in which pups are exposure to alcohol from postnatal days (PD) 4–9 in a binge-like alcohol exposure targets the growth spurt of the hippocampus and prefrontal that occurs during the third trimester of pregnancy. This model results in decreased the survival of newly generated cells in the adult rat hippocampus.\textsuperscript{17} Specifically, rats were administered bromodeoxyuridine (BrdU), a drug that labels actively proliferating cells, every other day from PD30–50. When the number of BrdU+ cells was assessed on PD80, 30 d following the injections, there were significantly fewer BrdU+ cells surviving in the alcohol-exposed group compared with controls. This reduction in surviving cells was replicated in a study where the number of BrdU+ cells was estimated following single injections of a thymidine analog on either PD42 or 80.\textsuperscript{15,16,19} In all these experiments, no alcohol-induced changes in cell proliferation were observed either through analysis of BrdU+ cell number 2 hours following injection or number of cells labeled with the endogenous protein Ki-67. In another study, a single day binge of alcohol on PD7 was sufficient to significantly decreased the number of adult-born cells that survived in adult mice (PD147) as measured by labeling with DCX and BrdU. Together, this body of work suggests that alcohol exposure during hippocampal development can permanently disrupt neuronal maturation and synaptic integration. More work is needed to determine signaling pathways that could be contributing to these effects.

It is important to note that several studies did not demonstrate an effect of developmental alcohol exposure on adult neurogenesis.\textsuperscript{17,18,20-23} As mentioned previously, several factors including timing of alcohol exposure, blood alcohol concentration achieved, and methods of analyzing adult neurogenesis could contribute to the different results reported across the literature. Genetic differences between strains that alter susceptibility or resilience to alcohol pathogenesis might also contribute. It is likely that alterations to hippocampal adult neurogenesis coincide with or are caused by dysfunction of other neuroplastic processes or signaling pathways as part of a broad pattern of central nervous system damage caused by developmental alcohol exposure. Thus, the lack of direct impact on adult neurogenesis does not imply a healthy brain or indicate that other neuroplastic processes are not affected. Systematic research is needed to answer these questions and identify molecular pathways that might be involved in alcohol-induced deficits in neurogenic processes.

**Immature neurons and dendritic complexity in rodent models of FASD**

A limited number of studies have investigated the effect of alcohol exposure on intermediate stages of cell differentiation and maturation. Gil-Mohapel and colleagues\textsuperscript{18} reported increased basic helix-loop-helix transcription factor NeuroD expression in the adult rat hippocampus following a perinatal exposure model
(all 3 trimester-equivalent). This increase in NeuroD+ cells could indicate delayed or stalled neuronal maturation in this model of FASD, a phenomenon that has been reported in in vitro models. In contrast, previous work from our research group has reported no change in the number of DCX+ cells in the adult rat hippocampus following PD4–9 alcohol exposure. Combined with our findings that demonstrate impaired cell survival in alcohol-exposed rats 30 d following a BrdU injection, it is likely that the reduction in neuronal survival in alcohol-exposed animals takes place soon after the cells have stopped expressing DCX. Ultimately, an inability of the hippocampus to place soon after the cells have stopped expressing DCX. Ultimately, an inability of the hippocampus to produce and integrate the number of new granule cells necessary for new memory formation could contribute to the cognitive deficits in children with FASDs and the behavioral impairments observed in animal models.

Our recent publication explored whether third trimester-equivalent alcohol exposure (PD4–9, 5.25 g/kg/day via intragastric intubation) altered dendritic morphology of immature (DCX+) granule cells in the adult rat dorsal dentate gyrus. We specifically targeted immature neurons which displayed significant neurite outgrowth, as these cells represented the oldest population of DCX+ neurons (Fig. 2A). These cells would soon progress through competitive integration and survival, during which the formation of a sufficient number of functional, excitatory synapses is necessary for the neuron’s continued survival. Our work demonstrated that PD4–9 alcohol exposure significantly decreased dendritic complexity of adult-born immature granule cells when measured on PD72. Using the 3D Sholl analysis, which places concentric spheres extending from the cell soma at set radii, we found that neonatal alcohol exposure was associated with reductions in total dendritic material (length) per radius (Fig. 2B), number of dendritic intersections at each radius (Fig. 2C), and the number of bifurcations per radius (Fig. 2D). Simpler dendritic structure could impair the ability of the cells to make a sufficient number of synaptic connections, hinting at a possible mechanism contributing to the previously reported decreased long-term cell survival reported in alcohol-exposed animals. Interestingly, when the numbers of progenitor cells (Ki-67+; Fig. 2E) and immature (DCX+; Fig. 2F) neurons in the dorsal dentate gyrus were assessed on PD72 using unbiased stereology, there were no significant treatment-related changes found, replicating our previous findings, as well as supporting the hypothesis that this model of alcohol exposure does not alter new cell generation or the initial stages of maturation. Instead, our model of FASD targets the later stages of granule cell maturation.

Previous work from our research group and others consistently report changes to dendritic morphology and spine density in late-developing structures such as the prefrontal cortex and hippocampus in rodent models of FASD. In the dentate gyrus, chronic alcohol exposure in adult rats decreased dendritic length of granule cells. Shorter exposures (1–4 weeks) also decreased dendritic complexity of immature neurons in the adult dentate gyrus. Specifically, the number of dendritic endings and total length was decreased following all alcohol exposure paradigms. Changes to dendritic morphology of mature neurons could be indicative of disrupted hippocampal connectivity and circuit dysfunction.

Administration of alcohol to cultured CA1 pyramidal cells decreased dendritic length and number of dendrites per cell. Spine density of CA1 neurons is not affected by prenatal alcohol in rats housed in isolation; however, alcohol-exposed rats housed in a complex “enriched” environment (which increases spine density in normal animals) display no change in spine density compared with animals housed in standard conditions. These findings suggest that alcohol exposure might produce “loss of function” deficits or latent impairments that only emerge when the system is challenged in some way. This hypothesis is also supported by evidence that exercise robustly increases cell proliferation in the dentate gyrus of normal rats that persists for up to 30 d after access to wheel running ceases. Conversely, while there is no baseline reduction in cell proliferation and exercise initially increases new cell generation in alcohol-exposed rats, this enhancement is no longer present 30 d later as seen in control animals.

Outside of the hippocampus, alcohol-induced changes to dendritic morphology have also been reported in other regions. Perinatal alcohol exposure (combining pre- and postnatal exposure) had limited effect on dendritic structure in the nucleus accumbens, but reduced spine density on Layer II/III pyramidal cell dendrites in the medial prefrontal cortex. PD4–9 alcohol exposure negatively impacts basilar dendritic complexity in Layer II/III pyramidal neurons of the medial prefrontal cortex. Spine morphology on the basilar dendrites shifted to a more mature, less plastic
phenotype, though spine density was unchanged. On the other hand, the apical dendrites are affected in an almost opposite pattern by PD4–9 alcohol exposure: dendritic tree morphology was stable but spine density was significantly decreased in alcohol-exposed animals.33 Similar results were found following PD2–6 exposure via vapor inhalation: Layer II/III basilar dendrites had a simplified structure compared with controls.34 Based on these findings, it is possible that prefrontal cortex communication with subcortical structures, such as thalamic nuclei, is impaired in alcohol-exposed animals. The exact mechanism through which developmental alcohol exposure compromises dendritic complexity in the adult brain is not well understood, but it is thought that these alterations contribute significantly to cognitive deficits in children with FAS. Overall, the abnormalities in dendritic morphology and spine density in alcohol-exposed animals likely contribute significantly to the behavioral deficits observed in rodent models of FASDs.

**Figure 2.** Third trimester-equivalent alcohol exposure (PD4–9; 5.25 g/kg/day) alters dendritic complexity of DCX+ cells in the PD72 rat dorsal dentate gyrus without affecting the number of Ki-67+ and DCX+ cells. A) Representation of DCX+ immature neurons in the dentate gyrus traced and used for Sholl analysis (40x lens). Alcohol-exposed animals had significantly less dendritic material per radius (B), fewer dendritic intersections (C), and fewer bifurcations per radius (D) compared with controls. Significant differences (p < 0.05) at each radius are indicated as a = AE vs. SC, b = AE vs. SI, and c = SI vs. SC. Number of Ki-67+ progenitor cells (E) and DCX+ immature granule cells (F) did not differ between the neonatal conditions on PD72. AE: alcohol-exposed, SI: sham-intubated, SC: suckle control. All values are means ± SEM. Fig. 2A–D adapted with permission from Boschen et al., 201624 (see Fig. 2, 4–6).

**Exercise and environmental complexity benefit the alcohol-exposed brain**

Understanding the effects of developmental alcohol exposure on hippocampal adult neurogenesis and overall hippocampal function is necessary in order for the development of effective behavioral and
pharmaceutical therapies. Our research group has investigated the impact of exercise and exposure to a complex environment on the alcohol-exposed rat brain. Our model of exercise uses a cage of 3 rats with 24 hr access to a stainless steel running wheel. The rats can run in the wheel either separately or together, and, in general, run ~3 miles per 24 hr period. The environmental complexity paradigm consists of 9–12 rats housed per cage for 30 d. The cage has 3 floors and an assortment of enrichment items (tunnels, balls, igloos, blocks) which are changed out for novel items every 2 d. We have reported that 12 d of wheel running followed by 30 d of housing in environmental complexity (WREC) brings the rate of survival of adult-born neurons in the postnatally alcohol-exposed rat hippocampus back to control levels.\textsuperscript{16,35} In addition, WREC returns behavioral performance (that demonstrated deficits in hippocampal-associated tasks) to control levels.\textsuperscript{36,35} However, Choi and colleagues\textsuperscript{37} reported that moderate prenatal alcohol exposure prevented mice from showing a robust neurogenic response to housing in EC. Differences in species, alcohol exposure window, or EC paradigm could explain discrepancies in the results between these 2 studies. The “priming” of alcohol-exposed rats with the experience of enhanced activity (running in the wheel) could possibly make them more susceptible to the stimuli in the EC. Our recent study used both WREC and 42 of continuous access to wheel running (WRWR) as behavioral interventions for postnatal alcohol exposure.\textsuperscript{24} We reported that both WREC and WRWR dramatically enhanced dendritic complexity of immature dentate gyrus neurons in PD4–9 alcohol-exposed adult rats. The beneficial influence of WREC on dendritic morphology could explain how this intervention enhances long-term cell survival.\textsuperscript{16} Other studies have also demonstrated the positive effect of exercise on hippocampal adult neurogenesis and dendritic complexity.\textsuperscript{38,39,40,41,42,43,44}

Both interventions likely work, to a large extent, through similar mechanisms to alter dendritic morphology and promote cell survival: transient increases in neurotrophic factors, angiogenesis, and hippocampal circuit activation. Our study\textsuperscript{24} showed that WRWR strongly increased levels of the neurotrophin brain-derived neurotrophic factor (BDNF), a molecule critical for cell maturation and neuroplasticity. Intriguingly, BDNF was not enhanced following WREC in our experiment, despite the powerful positive benefit seen on dendritic complexity. Recent work has suggested that EC promotes immature neuron maturation in the dentate gyrus through the activation of parvalbumin-positive interneurons by mature granule cells.\textsuperscript{45} Contrary to its action in the adult brain, GABA has an excitatory influence on neural progenitors and immature neurons and is critical for proper neuronal maturation. GABAergic stimulation of immature neurons by this population of interneurons prepares these cells of receive excitatory input from the entorhinal cortex, enhancing their long-term survival potential. Both empirical evidence and the ability to easily translate these interventions for use in humans support the further investigation of exercise and environmental complexity as behavioral therapies for children with FASDs.

**Conclusion**

FASDs place a heavy physical, emotional, and monetary burden on the affected individual, their immediate families and society as a whole. Uncovering the distinct ways that prenatal alcohol exposure impacts cognition across the lifespan is a key step in developing appropriate therapeutic interventions to help individuals with FASDs live independent and fulfilling lives. The evidence presented in this review suggests alcohol-induced deficits in hippocampal adult neurogenesis and dendritic morphology as possible contributors to memory and cognitive impairments observed in animal models of FASDs. Adult neurogenesis and dendritic outgrowth are dynamic, complex processes that require a precise balance of neurochemical signaling. Alcohol administration during key developmental time points disrupts the ability of newly generated hippocampal cells to successfully mature and form stable, functional connections long after the alcohol exposure has ceased. However, current literature on this topic is mixed, and more research is needed to determine genetic and environmental factors that account for this variability. In addition, further work is needed to extend these results to the clinical population. Once the exact nature of alcohol’s disruptive influence on adult neurogenesis and dendritic structure are understood, specific pharmacological treatments can be developed. Until then, exercise and targeted cognitive therapies represent promising therapeutic avenues to manage the behavioral symptoms of FASDs, in part through their beneficial effect on adult neurogenesis and general hippocampal plasticity.
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ORCID

Anna Y. Klintsova http://orcid.org/0000-0003-0626-8385

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