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Animal Models of Fetal Alcohol Spectrum Disorders: Impact of the Social Environment

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Abstract

Animal models of fetal alcohol spectrum disorder (FASD) have been used to demonstrate the specificity of alcohol's teratogenic effects and some of the underlying changes in the central nervous system (CNS) and, more recently, to explore ways to ameliorate the effects of alcohol. The main point of this review is to highlight research findings from the animal literature which point to the impact of the social context or social behavior on the effect(s) of alcohol exposure during development, and also to point to research questions about the social environment and effects of prenatal alcohol exposure that remain to be answered. Alcohol exposure during early development alters maternal responding to the exposed pup in a variety of ways and the alteration in maternal responding could alter later stress responsivity and adult maternal and social behavior of the exposed offspring. Environmental enrichment and voluntary exercise have been shown to ameliorate some of alcohol's impact during development, but the roles of enhanced social interactions in the case of enrichment and of social housing during voluntary exercise need to be more fully delineated. Similarly, the role of social context across the lifespan, such as social housing, social experiences, and contact with siblings, needs further study. Because of findings that alcohol during development alters DNA methylation patterns and that there are alterations in the maternal care of the alcohol-exposed offspring, epigenetic effects and their relationship to social behavior in animal models of FASD are likely to become a fruitful area of research. Because of the simpler social behavior and the short lifespan of rodents, animal models of FASD can be useful in determining how the social context impacts the effects of alcohol exposure during development.

Keywords

fetal alcohol spectrum disorder; rat; environmental enrichment; social behavior; epigenetics; maternal; voluntary exercise

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Fetal alcohol spectrum disorders (FASDs) involve wide-ranging deficits in growth, anatomy, behavior, and cognition [Sokol et al., 2003; Nash et al., 2006; Kodituwakku, 2007; Spadoni et al., 2007]. Fetal alcohol syndrome (FAS), the most severe of the FASDs, includes prenatal and/or postnatal growth retardation, CNS dysfunction with or without

obvious brain malformation, and a defining pattern of craniofacial malformations [Sokol et al., 2003; Bertrand et al., 2005; Hoyme et al., 2005]. The combined incidence of all FASDs (e.g., FAS, alcohol-related neurodevelopmental disorders (ARNDs), etc.) ranges up to 10 per 1,000 live births [Sampson et al., 1997; May and Gossage, 2001; O'Leary, 2004] with a higher incidence among certain groups depending upon social, demographic, behavioral, and other medical risk factors [Abel, 1995; May et al., 2007, 2008]. The functional expression of the CNS consequences of prenatal alcohol exposure includes cognitive and learning disabilities, poor attentional processes and hyperactivity, mental retardation, and behavioral problems [Kodituwakku, 2007].

Many of the behavioral problems in FASD seem to concentrate around poor social skills and inappropriate social interactions [Streissguth et al., 1991] that are clearly discriminated from the autism spectrum disorders [Bishop et al., 2007]. Using the Vineland Adaptive Behavior Scales, Thomas et al. [1998b] found that children with FAS were most severely impaired in interpersonal relationship skills and that these social deficits were not simply due to low intelligence quotient (IQ) but may reflect an arrested development of social ability. Some of the key social behavioral problems identifying children or teens with FASDs, using a scale specifically constructed for that purpose, are that they are chatty "but say little" or say things that seem out of context, overreact to situations with inappropriately strong emotional responses, demand attention or monopolize conversations, are unaware of the consequences of actions, interrupt others, are "overly friendly," and do not seem able to "take a hint" [Streissguth et al., 1998], all of which imply at least poor sensitivity to subtle social cues. Children with FASD may be more likely to lie [Rasmussen et al., 2008] and appear to have a deficit in moral judgment specific to social relationships [Schonfeld et al., 2005]. They are also vulnerable to language and social communication deficits which leads to atypical social interactions or adverse social experiences [Coggins et al., 2007]. The poor executive functioning in FASD contributes substantively to poor social behavior and to difficulties handling social situations [Schonfeld et al., 2006; McGee et al., 2008]. However, the disrupted attachment behavior in infants born to women who were social drinkers suggests that either prenatal alcohol exposure or the maternal responding to the infant may contribute to disruption in the maternal-infant dyad prior to a prominent role of executive functioning in social behavior [O'Connor et al., 1992].

Historically, animal models of FASD have been critical to our understanding of FASD, with early research being pivotal in proving that alcohol was indeed the culprit teratogen and clearly linking behavioral deficits to alcohol-induced changes in the CNS. Early animal models using rodents (mostly mice and rats) focused on the effects of exposure to alcohol during the prenatal period. However, it was known that while the development of rodents followed the same fundamental sequence as that in humans, the timing of the events of brain development differed relative to birth such that the early neonatal period just after birth in the rat was equivalent to the third trimester in humans [Bayer et al., 1993]. The period of brain development comparable to third trimester is a phase when the brain is undergoing rapid growth and is vulnerable to the impact of alcohol [West, 1987; Tran et al., 2000]. Thus, current rodent models of FASD may entail exposure to alcohol during the prenatal period, postnatal period, or the combination of the two. Animal models of FASD allow for the control of nutritional, environmental, and genetic factors to a degree that is not possible in studies on the human population.

Because animal models of FASD vary with respect to timing of alcohol exposure, dose and pattern of alcohol exposure, and mode of administration of alcohol, the findings from these models are not always consistent. Nevertheless, there has been good general correspondence between the behavioral effects of alcohol exposure during development found in a variety of animal models and the clinical condition of FASD [for reviews, see Driscoll et al., 1990;

Kelly et al., 2000]. With respect to how alcohol exposure impacts the brain during development, there is also general agreement that the hippocampus, cerebellum, and the hypothalamic-pituitary axis are impacted in a variety of models [e.g., Tran and Kelly, 2003; Tran et al., 2007; Johnson et al., 2008; Weinberg et al., 2008]. Nevertheless the timing of the alcohol exposure [see for example, Thomas et al., 1996, 1998a; Goodlett and Johnson, 1997; Maier et al., 1997, 1999; Tran et al., 2000; Cronise et al., 2001; Tran and Kelly, 2003] clearly impacts whether and how specific behavioral and brain deficits are seen. It is likely that this variability in findings from animal models in FASD is paralleled by variable findings in the clinical research resulting from differences across individuals with respect to exposure to alcohol. This review focuses on another potential reason for variable findings in both the clinical and animal research, namely that of social context.

Recently, the focus of research on FASD has shifted appropriately to treatment and interventions in FASD and research using animal models of FASD still plays a critical role because of the ability to better control socio-environmental and developmental factors and conduct studies quickly across generations. In this brief review, we focus on how the social aspects of postnatal experiences, or social aspects of interventions such as environmental enrichment and voluntary exercise, may impact the outcome in animal models of FASD. Of particular importance in this review is the potential for the manipulation of the postnatal social environment to exert a strong influence on outcomes leading to possible amelioration or exacerbation of perinatal alcohol-induced deficits, including potential trans-generational effects.

Understanding these effects of social context could lead to targeted treatments, interventions, and prevention of the deleterious effects of alcohol exposure during development.

MATERNAL-INFANT INTERACTIONS IN ANIMAL MODELS

Attachment behaviors of infants born to women who are social drinkers are disrupted [O'Connor et al., 1992, 2002]. These studies on two different populations clearly show that in maternal- infant dyads in which there was heavy alcohol exposure during gestation, the infant displays an increase in negative affect and less attachment security and the mother displays less supportive behavior [O'Connor et al., 1992, 2002]. Because of the inability to control the composition of the mother-infant dyad, it is not clear whether these changes in the behaviors of both the mother and infant are due initially to alcohol-induced or alcohol-related (e.g., genetic) effects in the infant, some inherent differences in mothers who engage in social drinking during pregnancy, or a synergy between the two. Experimental studies using animal models can help address these issues. Maternal behavior has been studied intensively in the rat in particular [for reviews, see Numan and Sheehan, 1997; Fleming et al., 1999, 2002] and is the result of a complex interplay of hormonal milieu of the dam and the behavior of both the pup and dam. Disruption of the behavior in the maternal- pup dyad is known to have a critical impact on the long-term outcome of the pup and, in particular, on the socio-emotional behavior of the pup [Meaney, 2001; Champagne and Meaney, 2007].

Early research using rodent models of FASD focused on behavior of the alcohol-exposed pup. Rat pups exposed to alcohol during perinatal period clearly demonstrated decreases in the latency to attach to a nipple and decreased nipple-shifting [Chen et al., 1982; Rockwood and Riley, 1986, 1990; Barron et al., 1991], behaviors critical to the functioning of the maternal-pup dyad as well as to providing nutrition for the pups. Another critical aspect of pup behavior is the ultrasonic vocalizations upon separation from their dam; these vocalizations regulate both maternal hormones and behavior [Hashimoto et al., 2001]. The frequency of ultrasonic vocalizations has been proposed to be a measure of separation

distress and increases with increasing amounts of separation distress [see Hofer et al., 1996, 2002 for reviews]. Ultrasonic vocalization have been shown to be reduced by prenatal alcohol exposure when tested on postnatal day 15 [Kehoe and Shoemaker, 1991] [although see Zimmerberg et al., 1995], and by postnatal alcohol exposure when tested on postnatal day 10 [Barron et al., 2000]. Surprisingly, these ultrasonic vocalizations are increased by exposure during the combined prenatal and postnatal period on postnatal day 5. In an attempt to resolve the discrepancy, a time course of the changes in vocalizations was examined. As can be seen in Figure 1, alcohol exposure during the combined prenatal and postnatal period clearly alters the pattern of ultrasonic vocalizations such that they are increased early in the postnatal period and then decreased in the later postnatal period. The ultrasonic vocalization data during the early postnatal period were collected 23 hr after the last alcohol administration, ruling out the possibility of intoxication contributing to the findings. The possibility of withdrawal contributing to the increased vocalization by the alcohol-exposed pups on postnatal day 5 cannot be ruled out completely. However, withdrawal cannot be the sole explanation of the increased vocalizations in the alcohol-exposed animals because the increase does not occur on all postnatal days whereas withdrawal would. Also, in a previous study [Marino et al., 2002], the increased vocalizations in the alcohol-exposed pups was very sensitive to social context, which would not be likely if they were simply a withdrawal symptom.

The alterations in the time course of distress ultrasonic vocalizations in rats exposed to alcohol during development would likely have an impact on maternal behavior and physiology. However, to elucidate the nature of these changes, it is important to examine them in rat dams that have not been exposed previously to alcohol, which entails cross-fostering pups from a treated to a non-treated dam. Cross-fostering itself can disrupt behavior [Ackerman et al., 1977; Misanin et al., 1977] so the design and interpretation of such studies is difficult and requires an extensive number of groups. Nevertheless, disruptions of the maternal behavior have been shown to be present in dams not exposed to alcohol and given cross-fostered ethanol-exposed pups [Fernandez et al., 1983; Marino et al., 2002]. Importantly, untreated dams with cross-fostered pups that had been exposed to ethanol during the combined prenatal and postnatal period show a greater amount of arched-back nursing when the pups were not intoxicated [Marino et al., 2002], a maternal behavior clearly related to later stress responsivity in the pups [Francis et al., 1999]. In addition, some studies examining maternal behavior in response to pups exposed to alcohol during the prenatal period only have suggested that there are alterations in the maternal responses to the pups [Fernandez et al., 1983; Ness and Franchina, 1990; Subramanian, 1992] although arched-back nursing was not specifically examined in these early studies. Together, the research suggests that it is possible that the increased responsivity to stress seen in alcohol-exposed rodents is not a direct teratogenic effect but instead one mediated by or secondary to changes in maternal behavior or the result of a synergy between teratogenic effects and abnormalities in maternal behavior.

Importantly, the maternal behavior of female alcohol-exposed offspring is also disrupted such that there are deficits in their ability to retrieve pups and to respond maternally to pups when naïve [Barron and Riley, 1985; Wilson et al., 1996]. There are also deficits in the ability to display paternal behavior in ethanol-exposed adolescent males [Barron and Riley, 1985; Wilson et al., 1996] but not adult males [Barron and Riley, 1985]. Disruptions in maternal behavior could be the result alcohol-induced changes in brain or hormone levels [e.g., Wilson et al., 1996]. However, it is known that maternal behavior of the adult organism is influenced by the maternal behavior that was experienced by the organism as a neonate [Fleming et al., 2002]. Thus, it is also possible that the observed disruption in the maternal behavior of rats exposed to alcohol during the perinatal period [Barron and Riley,

1985; Wilson et al., 1996] may be mediated in part by differences in the maternal behavior that was directed toward these alcohol-exposed rats during the early postnatal period.

In summary, there is substantial evidence that the maternal environment of the alcohol-exposed offspring is altered which may impact the development either separately or interactively (synergistically) with a teratogenic effect of alcohol. Because of studies using cross-fostering and/or just prenatal exposure, the evidence suggests that the initial cause of the alterations in the maternal-infant dyad in animal models is due to alterations in the exposed pup's behavior. Nevertheless, there is the potential for manipulations of maternal responsivity to moderate the impact of alcohol on the offspring either positively or negatively. Finally, the maternal behavior of the alcohol-exposed offspring is also altered suggesting the potential for multi-generational effects (see below for more discussion of this point).

ENVIRONMENTAL ENRICHMENT

We recently detailed some of the effective ways postnatal experiences and the environment has been manipulated to ameliorate the neurobehavioral sequelae of prenatal or neonatal alcohol exposure in rodent models of FASDs [Hannigan et al., 2007]. These manipulations have included neonatal handling which involves removal of the rodent from the nest and exposing it to human handling during the early postnatal period [e.g., Weinberg et al., 1995], environmental enrichment which typically involves housing several conspecifics with various objects in an arena much larger than a typical cage [e.g., Hannigan et al., 1993], voluntary physical exercise in running wheels [e.g., Klintsova et al., 1998, 2002a], and motor training in challenging motor tasks such as walking on a thin rod [e.g., Klintsova et al., 1998]. All of these manipulations include de facto alterations in the social environment and the prior review had not emphasized the role that social interactions, or the lack of such interactions, among animals may play in the beneficial effects of those experience [Hannigan et al., 2007]. One of the strengths of the classical “environmental enrichment” paradigm is that it is a global manipulation including exposure to a larger space, more objects, more physical challenges, and more opportunities for social interactions compared to that of the standard group-housed rodent caging of 3 rats in a cage. The social differences are that the enriched groups are reared for 8 or more weeks in same-sex groups of ~12 rats in large arenas filled with various objects the animals could play on, manipulate, chew, and crawl into. This treatment group contrasts sharply with another control group that in a typical design is comprised of rats which are housed in social isolation in steel/wire cages after weaning.

Discriminating the specific effects of varying social interactions within global environmental enrichment may have important implications for treatment of children who are characterized consistently as having poor social skills [e.g., O'Connor et al., 2006; Schonfeld et al., 2009]. The impact of the social enhancement or deprivation relative to the standard housing condition in ameliorating or exacerbating neurobehavioral effects of prenatal alcohol exposure could not be discriminated from the other aspects of the global environmental “enrichment” in this previous animal research. In the first studies of enrichment after prenatal alcohol exposure in rats or mice [Hannigan et al., 1993; Wainwright et al., 1993; Berman et al., 1996], there were no standard housing conditions so key comparisons were limited to the interaction between prenatal alcohol exposure and responses to enrichment versus social isolation. This allowed, as an alternate interpretation to the ameliorative effects of enrichment, that the postweaning social isolation may have led to hindlimb gait dysfunction and poorer performance in a spatial learning task [Hannigan et al., 1993; Wainwright et al., 1993]. Similarly, this design limited conclusions about how prenatal alcohol exposure reduced changes in neuroanatomical plasticity in hippocampus

[Berman and Hannigan, 2000], and perhaps neocortical thickness [Wainwright et al., 1993], in response to environmental enrichment in comparison with social isolated groups. Further studies including a group housed with multiple conspecifics but without exposure to a large arena with multiple objects are needed to elaborate the specific role of social interactions within the typical enrichment environment which includes exposure to a large arena and multiple objects in addition to multiple conspecifics.

One possible explanation for the relative failure of prenatal alcohol-exposed animals to show increases in dendritic spine density [Berman et al., 1996] or cortical thickness [Wainwright et al., 1993] in response to enrichment is that alcohol-exposed rodents do not respond to the enrichment the same way that nonexposed animals do. We may ask, in particular, if the “enriched” rats are actually engaging in the social interactions thought to be beneficial. To test this, we examined social interactions of identified individual animals housed in the enriched condition. Each arena included 12–15 same-sex animals, 4 or 5 from each of the three prenatal treatment groups—control, sucrose, or ethanol-exposed. Individual animals were uniquely marked, the group videotaped for 1 hr each under lighted and darkened (black-light) conditions, and social behaviors scored, including play, investigation/sniffing, allo-grooming, fighting, etc. There were differences in general levels of activity between light and dark conditions, and between males and females, in proportion of time spent of fighting, but there were no significant differences among prenatal treatment groups in the social behavior, regardless which prenatal group or groups were represented in the paired interactions [Mathison and Hannigan, unpublished findings]. Given other findings of abnormal social interactions in alcohol-exposed rats [for a review, see Kelly et al., 2000], these findings suggest that the alcohol-induced changes in social interactions are context-specific. However, with respect to the environmental enrichment context, it still remains to be determined whether the impact of the social interactions differs across treatment groups.

There are clear implications of the social aspects of environmental enrichment for children with FASDs. First, it is premature to conclude that filling an affected child’s life with more and more varied experiences would be beneficial. In fact, some of the first studies of FASD children in school settings, which can certainly be considered “socially stimulating,” in term of numbers alone, compared to home, reported the children responded poorly to the increased stimulation. Educators working with these children recommended setting aside private workspaces with fewer distractions to help mitigate this reaction to “enrichment.” There is also considerable evidence from the testimonies of family and teachers to support this conclusion [e.g., several chapters in Kleinfeld and Wescott, 1993]. Finally, O’Connor and others have examined the impact of social skills training and have suggested that this type of training is effective as long as the children with FASDs have relatively intact executive functioning [Floyd et al., 2006; Frankel et al., 2006; O’Connor et al., 2006; Schonfeld et al., 2009]. The literature on environmental enrichment in rodent models of FASD and treatment of individuals with FASD suggests that enrichment and other treatment interventions must be designed with the capability of the individual in mind and people with FASDs often have limitations on their cognitive ability and may well have limitations on their ability to process the sensory stimulation of enrichment.

VOLUNTARY EXERCISE

Although social interactions are a key component of environmental enrichment studies, it is perhaps less appreciated that social context is also likely an important consideration for the effectiveness of interventions involving voluntary exercise or other forms of physical training such as acquisition of new motor skills [Klintsova et al., 1998, 2002]. For example, recent studies have shown that housing normal adult rats in social isolation while they are given opportunities to engage in wheel-running retards the typical stimulation of adult

neurogenesis in the hippocampal dentate gyrus (labeled with 5-bromo-2'-deoxyuridine (BrdU)), relative to group-housed rats [Stranahan et al., 2006; Leasure and Decker, 2009]. The reduced effectiveness of exercise in stimulating adult neurogenesis under social isolation has important implications for the potential use of exercise therapy, to the extent that rehabilitative effects of exercise are linked, at least in part, to the increased production and survival of newly generated neurons in the dentate gyrus. For example, offspring of rat dams consuming ethanol in a liquid diet during the prenatal period showed significantly decreased proliferation and survival of adult-generated BrdU-labeled dentate gyrus cells compared to ad lib chow control groups. Notably, the offspring of dams given pair feeding of the liquid diet alone also had significant reductions in survival (though not proliferation) of adult-generated cells compared to controls. However, voluntary wheel-running exercise significantly increased the number of BrdU-labeled cells that survived to the same degree in all three prenatal treatment groups [Redila et al., 2006]. The same investigators showed that voluntary exercise also reversed deficits in performance of a Morris water maze task and deficits in theta-patterned long-term potentiation in the hippocampal perforant path [Christie et al., 2005]. In those reports, the rats were housed individually from adolescence. Whether socially housed rats given prenatal alcohol exposure would show similar deficits (relative to controls), and similar reversal of those deficits with voluntary exercise, has not yet been tested.

In the neonatal rat model of alcohol exposure during the third trimester, we have shown that early postnatal binge-like alcohol exposure results in a persistent impairment in the number of newly generated neurons (generated in adolescence and young adulthood) that survive to full maturity, without an apparent deficit in the proliferation of the progenitor cells [Klintsova et al., 2007]. This raises the questions of whether some of the behavioral or cognitive deficits evident in this rat model may reflect abnormalities in acquisition of new neurons via adult neurogenesis and whether increased survival of newly-generated neurons in the dentate gyrus may provide a form of therapeutic rehabilitation. In addressing this question, we assessed whether wheel running during adolescence and early adulthood could stimulate hippocampal neurogenesis. In our first study, the rats were housed together during the 12-hr light phase of the light/dark cycle, when the running wheel was not available, and then placed individually in the wheel-running cages for 12 hr each day during the dark phase (permitting monitoring of individual running activity). Surprisingly, there was no significant increase in cell proliferation per se or in neurogenesis induced by the voluntary exercise [Klintsova, personal communication]. Around that time, Stranahan et al. [2006] published their study showing that social isolation delays the neurogenic effect of exercise. We reasoned that either the experience of running in isolation for 12 hr per day or the effect of daily cycles of running in isolation and returning to group housing was reducing the typical neurogenic response. In a follow-up study, we then modified the design so that animals were either housed 3 rats per cage with 24-hr access to the running wheel or were maintained 3 per cage without access to a running wheel. Under these conditions, wheel running did stimulate a neurogenic response in control and alcohol-treated rats, but the long-term survival of newly-generated neurons was still impaired in the alcohol-treated rats [Klintsova, personal communication]. On the other hand, a recent report from Thomas et al. [2008] showed that deficits in spatial learning and increased open field activity in rats given neonatal alcohol exposure could be ameliorated when tested shortly after 30 days of voluntary exercise with social housing compared to social housing alone from postnatal days 21 to 51. The effects of the voluntary exercise in the alcohol-exposed rats were of a similar magnitude to that observed in rats not exposed to alcohol. If confirmed, these findings suggest a role for experience-dependent increases in hippocampal neurogenesis in developing therapeutic interventions for FASDs.

As a caveat, the functional role of adult neurogenesis is still debated [Leuner et al., 2006], and its potential contribution to therapeutic rehabilitation of the alcohol-damaged brain, or even its potential adverse effects, is still uncertain. However, given the possibility that adult neurogenesis may provide one viable avenue to neuroplasticity and functional improvement, animal models will be very useful in advancing the understanding of conditions under which it may prove effective. The studies examining the social context, neurogenesis and voluntary exercise indicate that it will be particularly important to consider the social context when exploring ways to ameliorate the impact of alcohol exposure during development.

REACTIVITY TO SOCIO-EMOTIONAL STRESSORS

One of the factors that will clearly impact interventions and treatment for FASDs is the reactivity to stress. Differential increased reactivity to stressors was one of the earliest findings from valid animal models of FASDs [Weinberg and Bezio, 1987; Weinberg, 1988] and there has been recent evidence that this differential reactivity is seen in the cortisol response and in negative affect in response to a still face in infants exposed to alcohol during development [Haley et al., 2006]. The behavioral, physiological, and endocrine mechanisms that mediate stress reactivity and anxiety are also intimately involved in social behavior and social interactions. Manipulations of social situations are classic ways of inducing a stress response in an organism. In rodents, the social interaction test, which measures social behaviors in an unfamiliar context, is considered to be a standard test of anxiety [for a review see File and Seth, 2003]. In human infants, stress reactivity can be measured using the presentation of an unusual social stimulus, namely that of a still face [Ham and Tronick, 2006]. There has been a recent excellent review on how alcohol exposure during development impacts stress reactivity with a focus on the hypothalamic- pituitary axis and sex differences [Weinberg et al., 2008] and it is not the intent of this article to cover that material. Instead, we will focus on the interaction between alcohol-induced changes in reactivity to social stressors and social behavior.

Social stressors for rodents include isolation and various types of social interactions including defeat during an aggressive encounter and crowding in the home cage. Not surprisingly, these stressors have been shown to impact later social behaviors including aggression, sexual behavior, and social motivation [Valzelli and Garattini, 1972; Wongwitdecha and Marsden, 1996; van den Berg et al., 1999a; de Jong et al., 2005]. Isolation has repeatedly been shown to be a particularly severe stressor during adolescence [van den Berg et al., 1999b; Douglas et al., 2004]. However, controlled experiments examining the effects of isolation or exposure to aggressive encounters on the impact of alcohol exposure during development have not been conducted. It is known that alcohol-exposed animals show alterations in aggression although the nature of the alteration varies according to extent of alcohol exposure and species [see Kršiak et al., 1977; Brain et al., 1987; Lugo et al., 2006]. However, when nutrition is controlled and alcohol exposure occurs across the period equivalent to all of gestation in the human, inter-male aggression is reduced in animal models of FASD [Lugo Jr. et al., 2006] when the motivation for aggression is strong. This finding is consistent with previous findings of reduced investigation of other males by alcohol-exposed males [Kelly and Dillingham, 1994; Kelly and Tran, 1997] and suggests a reduction in territoriality in these animals. Repeated aggressive encounters and defeat result in a host of changes in brain and behavior, including increased drug intake [for a review see Miczek et al., 2008]. Given that alcohol exposure during development impacts aggressive behavior, it seems likely that repeated aggressive encounters would also have differential effects in alcohol-exposed animals and this area of research begs for more attention.

Little has been done examining the effect of siblings on the impact of alcohol exposure during development. The studies using prenatal alcohol exposure typically have all the animals in one litter treated similarly whereas often (although not always), studies using just postnatal alcohol exposure have pups that were treated differently in one litter. As far as we know, the make-up of the litter has not been varied systematically to determine its impact on the effect of alcohol exposure. It is known that variations in the size of the litter and the behavior of siblings impacts later behavior and that each litter has distinct characteristics in their huddling. Isolation during the adolescent period has been shown to have dramatic effects on play behavior and later social behavior but again, these manipulations have not been done using animal models of FASD. Interestingly, artificial rearing has been used as a means to administer alcohol during the early postnatal period by all of the authors of this review and we have observed that this procedure typically resulted in greater deficits than those observed after intubation [e.g., Goodlett and Johnson, 1997; Tran et al., 2000; O'Leary-Moore et al., 2006]. Whether the interaction of artificial rearing and alcohol is due to alternations to the social context (including maternal deprivation or isolation of the rat pups from their siblings) or changes in nutrition due to the artificial milk formula used in artificial rearing is not known at this time. Nevertheless, it is possible that social factors during early development may exacerbate the effects of alcohol during development. Likewise, none of the various methods of neonatal alcohol exposure in rats and mice (intubation, injection, vapor inhalation, artificial rearing) obviates a key problem inherent in such models, i.e., that alcohol treatment of pups produces effects on the pup behavior (e.g., motor activity, suckling, ultrasonic vocalizations) during the periods of intoxication and withdrawal that influence maternal interactions and other aspects of normal development. These effects of alterations in social context may interact with the direct effects of alcohol on the pup's CNS or produce persistent effects in addition to the pharmacological actions of alcohol.

PROSPECTS FOR STUDYING EPIGENETIC ASPECTS OF FASD-RELATED SOCIAL PROBLEMS

Epigenetics refers to the regulation of gene expression without altering the actual DNA of an organism. Gene expression can be regulated in a variety of ways including via micro RNAs, histone modification, chromatin remodeling and DNA methylation. It has been suggested that this regulation is particularly responsive to the environment and can be stable across generations [Cooney, 2007]. The possibility that alcohol exposure early in development can produce epigenetic alterations (e.g., modify histones or change DNA methylation patterns) that affect developmental regulation of gene expression in the conceptus, and thereby have long-lasting effects on phenotypic development, is now an active area of investigation (see below). Recently, there has also been increased attention paid to epigenetic mechanisms underlying transmission of changes in social behaviors, particularly maternal behaviors, and stress reactivity. Maternal behavior has been shown to impact global methylation patterns in the brain and also produce differential methylation of hypothalamic estrogen receptors [Cameron et al., 2008]. These changes have been shown to be stable across at least three generations [Champagne and Meaney, 2007; Cameron et al., 2008]. From this review, it is clear that alcohol exposure during development impacts social behaviors and stress reactivity of the rat pups. Importantly, maternal behavior in the dams caring for alcohol-exposed infants is disrupted [Fernandez et al., 1983; Marino et al., 2002] and the maternal behavior of the alcohol-exposed individual [Barron and Riley, 1985; Wilson et al., 1996] is also impacted. Thus, early alcohol exposure may produce epigenetic effects either because of direct actions on fetal/infant DNA or from changes resulting from altered maternal-infant (or other) behaviors early in life.

There is evidence that implicates epigenetic mechanisms underlying at least some of ethanol's effects during development. Haycock recently wrote an excellent review indicating that many of alcohol's effects during development, including effects of alcohol before conception and effects on male gametes, are consistent with an epigenetic effect [Haycock, 2009]. Alcohol has also been shown to interfere with a number of chemical processes that are critically involved in epigenetics [for a review, see Haycock, 2009]. There have been two recent published studies demonstrating epigenetic effects of alcohol exposure during development. In one, heavy alcohol consumption tended to result in decreased methylation of normally hypermethylated regions in human sperm DNA [Ouko et al., 2009]. In an animal model of FASD, alcohol exposure during the preimplantation stage alters DNA methylation at a specific region of the DNA in the placenta but not the embryos [Haycock and Ramsay, 2009].

There is also indirect evidence that alcohol exposure during development induces or alters epigenetic processes. Lam et al. [2000] have shown that the pups born to dams that had been exposed to ethanol in utero were smaller physically and had slower latencies to right when placed on their back. This second generation effect was not seen in pups whose sire was exposed to ethanol in utero. Thomas and her laboratory have shown some dramatic effects of choline in reversing some of the behavioral effects of ethanol exposure during development [Thomas et al., 2000, 2004; Ryan et al., 2008]. Importantly, choline is effective even when given after the cessation of ethanol exposure [Ryan et al., 2008] and has effects that last after the choline treatment has been terminated [Thomas et al., 2004a; Ryan et al., 2008]. The mechanism of the effect of choline in ameliorating alcohol's effects remains to be determined. Choline has very diverse effects on the CNS including effects on neurotrophic factors, dendritic trees and spines, the cholinergic system, and cell death [McCann et al., 2006; Zeisel, 2006]. However, one of the most dramatic effects of choline is on methylation patterns in the brain. Some researchers have concluded that it is these epigenetic effects, and in particular the alterations of the methylation pattern of the DNA that mediate choline's impact on learning and memory [Zeisel, 2006].

The mechanism(s) of a number of developmental disorders have been postulated to involve epigenetic processes. Particularly, childhood obesity and adult hypertension have both been associated with alterations in methylation of particular genes during development, which then in turn influence the development of the phenotype [Bogdarina et al., 2007; Cooney, 2007]. Strikingly, child abuse has been suggested to impact the methylation of neuron-specific glucocorticoid receptor in humans and this has been suggested to contribute to the increase in suicide and psychopathology seen in victims of child abuse [McGowan et al., 2009]. Alcohol exposure in adult organisms has clearly been demonstrated to alter DNA methylation in liver tissue and this may mediate the onset of liver disease [Shukla et al., 2008]. Given that ethanol can impact epigenetics [Shukla et al., 2008], that ethanol exposure during development impacts behaviors, including maternal behavior, maternal-infant interactions and stress reactivity, that have all been shown to be regulated via epigenetic mechanisms, and that trans-generational effects of ethanol exposure during development have been demonstrated [Lam et al., 2000], epigenetic analyses promise to be interesting and fruitful avenues of research on FASD.

SUMMARY AND CONCLUSIONS

Similar to what we had noted previously regarding amelioration of fetal alcohol effects via enrichment [Hannigan et al., 2007], findings from animal studies about social behavior and social context may not translate in any simple or straightforward manner to understanding the behavior of FASD children in schools or at home. Nevertheless, animal models allow for examination of the role of social context under simpler and more controlled conditions and

across generations. Some nonrodent animal models, using primates [Schneider et al., 2002] or ferrets [Filgueiras et al., 2009], for example, may capitalize on species differences in social behavior to further clarify interactions with perinatal alcohol exposure.

This review points to the importance of the likely alterations in the behavior of the maternal-infant dyad in animal models of FASD and suggests that the alterations are initiated by alcohol-induced alterations in the offspring. The alterations could explain in part other changes in behavior of the alcohol-exposed offspring, such as an increase in stress responsivity and adult maternal behavior. Both environmental enrichment and voluntary exercise have been shown to be potential treatments for some of the deficits seen in animal models of FASD and the role of social context in both of these interventions could be very important. Some of the potential epigenetic effects of alcohol exposure during development may be due to the direct effect of alcohol or due to alterations in the maternal-infant dyad; importantly, epigenetic effects have the potential to persist across generations.

Research using animal models of FASD will be able to continue to contribute to the field as a whole by allowing the study of multiple generations quickly and allowing the manipulation of the social context in a controlled manner. Finally, as various animal models to date have been successful in first identifying other teratogenic outcomes in animals and only later in people [e.g., renal anomalies, DeBeukelaer et al., 1977], auditory deficits [Church, 1987], spatial learning dysfunction [Blanchard et al., 1987], and eyeblink conditioning impairments [Stanton and Goodlett, 1998], there is promise that further research examining social factors and social behaviors in animals can lead to important findings in affected people as well.

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Fig. 1.

Ethanol exposure during the combined prenatal and postnatal period alters the pattern of the frequency of ultrasonic vocalizations in isolated rat pups over development (Kelly, Lugo, and Marino, unpublished findings). Rat pups were isolated from their dam and ultrasonic vocalizations were detected using a bat detector. The litter was the unit of analysis and no pup was tested more than twice over the testing period. There are 15–17 animals of approximately equal numbers of males and females per data point. There were no sex differences in the frequency of ultrasonic vocalizations. The stars indicate a significant difference between the ethanol-treated group and both control groups on that postnatal day. Error bars represent standard errors of the mean (SEMs).