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Effects of alcohol exposure during development on play behavior and c-Fos expression in response to play behavior

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Abstract

Developmental exposure to alcohol can produce characteristic physiological and cognitive deficits, often termed Fetal Alcohol Spectrum Disorder (FASD). More recently, social deficits have been shown to occur both in FASD and animal models of FASD; the behavioral and neural bases of these deficits remain to be determined. It was hypothesized that changes in sensory processing may in part underlie the social deficits seen in FASD. This study used a rat model of FASD and social play, a behavior critical to adult social functioning, to begin to examine this hypothesis. Somatosensory cues from dorsal contact to the nape of the neck, critical to the initiation of pinning, were systematically degraded by administration of different doses of xylocaine, a topical anesthetic. Neuronal activity after one hour of play was assessed by measurement of c-Fos immunoreactivity (IR) in different brain regions. Ethanol-exposed rats showed an increase frequency of pinning during social play and were more sensitive to the degradation of somatosensory cues compared to the control groups, suggesting difficulties in processing somatosensory cues. Neuronal activity in the somatosensory cortex induced by play was significantly decreased in the ethanol-exposed group compared to the non-treated group. The c-Fos IR in the nucleus accumbens was altered in a sexually dimorphic manner in the ethanol-exposed group. Thus, the behavioral and brain measures are consistent with the hypothesis that ethanol exposure during development induces alterations in social play via deficits in processing somatosensory cues that are important to social play.

Keywords

Fetal Alcohol Syndrome; play; somatosensory cortex; nucleus accumbens; ethanol; development; c-fos

Introduction

The teratogenic effects of alcohol have been known for over thirty years. In 1973, Smith and Jones first characterized Fetal Alcohol Syndrome (FAS) which included facial abnormalities, growth deficits and central nervous system impairment [22]. Fetal Alcohol Spectrum Disorder (FASD) describes individuals not meeting the criteria for FAS but with deficits linked to prenatal alcohol exposure [28,59,66]. While cognitive impairments including mental retardation due to alcohol have been widely reported [33,44,65], more recently there has been a focus on social impairments seen in individuals with FASD. People with FASD show

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difficulties in interpersonal relationships [68], abnormal play behavior [21], and insecure attachment [45]. Consistent with problems in the social domain, FASD individuals are characterized trouble with the law, largely resulting from crimes against persons [66]. The crimes against persons include inappropriate sexual behavior such as rape or masturbation in public, burglary, and assault [66].

Animal models of FASD provide simpler systems to evaluate the effects of perinatal alcohol exposure on complex behaviors and allow a detailed investigation of the neural bases of any observed deficits. Rodent models of FASD have been shown to detect similar impairments in cognitive and social functioning as those seen in people with FASD (for reviews, see [7,24]). With respect to social behaviors in rodents, alcohol exposure during development has been shown to produce alterations in aggression, social interactions, and maternal and sexual behaviors [23,26,60,77]. The underlying neural and behavioral bases of these social alterations remain to be determined.

A particularly interesting form of social behavior is play because it is a behavior seen across nearly all mammals [4,11,55]. Social play, specifically play fighting, is the most commonly reported form of play in mammals [55]. Isolation during adolescence increases the frequency of social play in that period and can decrease social interactions in adulthood [18,46,71]. Play fighting is critical to establishing social organization of groups of animals and the development of intra-specific communication [73]. The key factor of play fighting is for one partner to gain an advantage, usually by contacting the nape of the neck [54]. This dorsal contact can induce the contacted play partner to roll over on its back resulting in a pin. However, the contacted partner simultaneously attempts to avoid the dorsal contact and launch a counterattack as part of the play sequence. The frequency of play fighting in the rat peaks around postnatal day 30 to 40 and declines after puberty. Importantly, prenatal alcohol exposure has been shown to alter social play in juvenile rats in a sex-dependent manner. Specifically, ethanol-exposed males had fewer pins than control males, whereas ethanol-exposed females had more pins than control females [37].

While it is known that alcohol exposure during development alters play behavior, the mechanism of this effect is not understood. Social play behavior is influenced by visual, olfactory, auditory and somatosensory cues [72,53,56]. Evidence suggests that somatosensory cues may be particularly important. Aspiration of the parietal cortex, which includes the primary somatosensory cortex, induces a 65% decrease in pins [50]. Additionally, anesthetization of the nape of the neck reduces but does not eliminate the response to a dorsal contact [62]. Importantly, ethanol exposure during development has been shown to induce changes in sensory systems underlying play behavior. Perinatal ethanol exposure causes reductions in cell number in the somatosensory cortex, olfactory bulb, hippocampus and the vestibulocochlear ganglion [3,8,15,40,43,57] and changes in the electrophysiological characteristics of whisker barrels and the visual cortex have also been observed after exposure to alcohol during development [35,58]. In particular, the electrophysiological findings suggest both a reduction of sensitivity and reduced plasticity in neurons in the somatosensory cortex [58] and reduced selectivity and plasticity in the visual cortex [36]. Thus, alterations in processing sensory stimuli due to alterations in the neural substrate may underlie changes in social play observed in rats following ethanol exposure during development.

This study uses a three trimester model of FASD to investigate both the neural and behavioral mechanisms by which alcohol exposure during development alters play behavior. This model differs from many other models of FASD because alcohol is given during the period equivalent to all three trimesters in the human [2] and has been shown to produce effects that differ from more limited exposure models [5,70]. Our overall hypothesis is that developmental ethanol exposure leads to atypical social behaviors as a result of alterations in sensory processing. This

paper focuses on the contribution of somatosensory signals to changes in play behavior. While extrapolation from neural changes in one contributing neural system to the behavioral manifestation of a complex behavior such as play is fraught with possibility for error, we predict that alcohol exposure during development results in an increase in somatosensory thresholds in the nape of the neck. This increase in threshold will result in an increase in pinning because the response of an ethanol-exposed rat will only occur when the rat pair is in close proximity to each other and thus the response is most likely to be pinning rather than another play behavior [53]. Because of the reduced cell number in the somatosensory cortex [38,43,57], there will be less redundancy in the system and thus, it was predicted that the degradation of the somatosensory signals would have a greater effect in the ethanol-exposed rats compared to controls. In addition, immunoreactivity of the immediate early gene c-Fos, rapidly activated in response to a variety of sensory stimuli, will be examined after one hour of play. Regions of interest include somatosensory cortex, dorsal striatum, nucleus accumbens, and pre-limbic cortex [17,48,63]. It was hypothesized that perinatal alcohol exposure affects play behavior via altered somatosensory integration which will be manifested as a reduction in c-Fos activation.

Materials and Methods

Animal Model

Animal facilities were accredited by the American Association of Laboratory Animal Care (AALAC), and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina. All animals were housed in the animal colony of the Department of Psychology at the University of South Carolina. The colony was maintained at 22°C with a 12:12 hr light: dark cycle, with the light beginning at 0700 h.

The animal model of FAS was the three trimester model [5,70] which entails alcohol exposure via intragastric intubation during the prenatal and postnatal period in the rat [2]. The intubation procedure takes less than 2 min in both dams and pups and every effort is made to minimize the separation time of the pup from the dam. Female Long-Evans rats were housed with an experienced male over night and gestational day (GD) 1 was defined by the presence of sperm in the vaginal smear on the following morning. Pregnant female rats were singly-housed and assigned to one of three groups: ethanol-treated (ET), intubated control (IC), and non-treated control (NC). Prenatal treatments occurred from GD 1 through GD 22. ET dams were intubated using a stainless steel gavage tube with 4.5 g/kg of ethanol in 20 ml/kg of distilled water on a daily basis between 3:00 and 5:00 PM; food and water was available *ad libitum*. IC dams were intubated with maltose-dextrin made isocaloric to the ethanol solution in 20 ml/kg of distilled water on a daily basis and the IC dams were pair-fed to an ET dam of similar weight. NC dams were weighed daily. The day of birth was GD 23 and no treatments occurred on this first postnatal day (PD 1). Litters were culled to eight animals, four animals of each sex when possible. Postnatal treatments occurred from PD 2 through 10. ET rat pups were intubated using Intramedic PE 10 tubing with 3.0 g/kg of ethanol in 27.8 ml/kg of enriched milk formula [76] at 11:00 AM followed two hours later with 27.8 ml/kg of milk alone. IC pups were intubated twice daily but no solutions were administered. NC pups were weighed daily. Rats were weaned on PD 21 and housed in same sex pairs from the same litter.

Blood samples (10 µl) were taken from the dams on GD 20 three hours after ethanol or control intubation and from pups on PD 10 two hours after ethanol or control intubation via a nick to the end of the tail. The times were chosen because they assess maximum blood alcohol concentrations (BACs) in this model [32]. Blood samples along with standards generated at the time of blood sampling were frozen at -80°C until time of assay. BACs were analyzed using an enzymatic procedure [9].

Test of Play Behavior

On PD 27, one male and one female pair per litter were isolated in order to facilitate an increase in play behavior [49]. All behavioral testing was conducted in well-lit conditions during the light cycle between 10:00 AM and 2:00 PM. On PD 32 through 34, rats were habituated to the testing location and chamber; each rat was placed with its former cagemate into a clear Plexiglas cage with bedding for a total of five min. Then on each of PD 35 through 39, rats received four subcutaneous (sc) injections of either vehicle (saline), 0.5, 1.0, 2.0, and 4.0% xylocaine prior to testing [62]. The four injections of 0.05 ml each were given on the dorsal surface just posterior to the ears and approximately 0.5 cm from the midline and on the dorsal surface at the level of the clavicle about 1.0 cm from the midline bilaterally. Injections were given 15 min before a five min test session which was videotaped. The order of the anesthesia condition across test days was counterbalanced using a latin squares design and each rat received each dose once.

Tapes were scored blind to condition; the inter-rater reliability was over 0.90. Each pair of rats (which received the same treatment) was scored as a single unit. The number of times that a pin occurred was recorded. A pin was defined as occurring when one rat is on its dorsal surface with the other rat on top. The frequency of dorsal contacts was also recorded. A dorsal contact was defined as occurring when one animal makes contact with any portion of its ventral surface (i.e. mouth, paws) onto the dorsal surface of its partner (tails being excluded). In order to exclude investigatory responses, contacts in the anogenital region were not included in this category.

Measurement of c-Fos Immunoreactivity

On PD 42, rats were placed in the same testing apparatus and allowed to play for one hour. Immediately after this period, rats were deeply anesthetized with sodium pentobarbital and perfused intracardially with phosphate-buffered saline followed by 4% paraformaldehyde. The brains were removed, post-fixed overnight in 4% paraformaldehyde and then incubated in a 15% followed by a 30% sucrose solution. The brains were frozen at -80°C until sectioned. Two sections per brain area were kept; brain areas included the prefrontal (limbic) cortex, somatosensory cortex (shoulder/neck region), nucleus accumbens, and dorsal striatum.

Sections were labeled for *c-fos* gene expression as described in Fadel et al. [9]; all steps were conducted at room temperature. Briefly, sections were washed in Tris-buffered saline (TBS) and methanolic peroxide and then incubated overnight in polyclonal rabbit anti-Fos antibody (1:10,000; EMD Biosciences, San Diego, CA). After rinsing in TBS, the sections were incubated with biotinylated donkey anti-rabbit antibody (1:1,000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1.5 hour, rinsed again in TBS, incubated with peroxidase-conjugated streptavidin (1:1,600; Jackson ImmunoResearch Inc., West Grove, PA), and then rinsed again in TBS. The Fos-like immunoreactivity (c-Fos IR) was expressed using diaminobenzidine tetrahydrochloride reaction. Sections were mounted on gelled slides and cover-slipped.

Quantification of the Fos-like immunoreactivity was conducted using an MCID imaging system (Interfocus Europe, Inc.). Areas of interest were defined according to the rat brain atlas ([51] and included prefrontal cortex (PFC) ($1000 \times 1300 \mu\text{m}$) (plates 10-12), nucleus accumbens (NA) ($1000 \times 1300 \mu\text{m}$) (16-18), dorsal striatum (STR) ($1000 \times 1300 \mu\text{m}$) (16-18), and somatosensory cortex (shoulder) (SH1) ($2000 \times 2200 \mu\text{m}$) (48-52)). Sections could not always be matched exactly, but were counted if within 2 plates. Digital images of 2 representative sections per area were captured with a Photometrics Cool Snap camera attached to a Nikon Optiphot microscope. The MCID basic program was used to set intensity and morphology parameters and then c-Fos IR cells were counted automatically.

RESULTS

For all analyses of repeated measures, Greenhouse-Geisser adjusted degrees of freedom were used.

Physical Parameters

Dam body weights and numbers are shown in Table 1. A repeated measures analysis of variance (ANOVA) on body weights over gestational day indicated that there was no significant effect of group or interaction with gestational day with group.

Pup weights and numbers are shown in Table 2. A repeated measures ANOVA with group and sex as between factors and the pup body weights across PD 2 through 10, PD 21 and PD 30 as the repeated measures revealed significant interactions between body weights and sex ($F(10, 180) = 28.6, p < 0.001$) and body weights and group ($F(20, 180) = 4.1, p < 0.01$). The interaction of body weights and sex was due to the difference between male and female body weights getting larger over the course of development (see Table 2). The interaction of body weights and group was the result of a difference among all three groups on PD 2 which became a difference between the two control groups and the ET group by PD 10 which remained stable on PD 21 and 30. On PD 2, the ET group weighed less than the IC group which weighed less than the NC group (Simple main effect $F(2, 183) = 17.8, p < 0.001$, Tukey's HSD p 's < 0.05). On PD 10, 21 and 30, the ET group weighed less than the IC and NC groups which did not differ from each other (PD 10: simple main effect $F(2, 183) = 11.3, p < 0.001$; PD 21: simple main effect $F(2, 183) = 3.6, p < 0.05$; PD 30: simple main effect $F(2, 183) = 5.9, p < 0.005$, Tukey's HSD p 's < 0.05).

Average BACs and standard error of the means (SEMs) for the dams, male ET pups, and female ET pups were 380.2 ± 3.0 , 367.7 ± 16.3 , 395.1 ± 21.7 mg/dl respectively. Separate t -tests comparing male and female pups and pups and dams revealed no significant differences.

Play Behavior

Separate ANOVAs using order of anesthesia condition as a between factor and dose of xylocaine as a repeated measure on either number of pins or dorsal contacts revealed no significant effects of or interactions with order indicating that order of anesthesia condition had no significant impact on the data. The dependent measures of number of pins and number of dorsal contacts were analyzed using two-way ANOVAs with group and sex as between factors and anesthesia condition as the repeated measure.

Analyses of dorsal contacts revealed only a significant effect of anesthesia condition ($F(4,348) = 64.5, p < 0.001$) but no effects of or interactions with group or sex (see Figure 1). The main effect of anesthesia condition was because 4% xylocaine significantly reduced dorsal contacts in all groups (Bonferroni t -tests, p 's < 0.001). There were no significant effects of the other doses of xylocaine on dorsal contacts.

Because 4% xylocaine produced a decrease in dorsal contacts which makes any changes in pinning difficult to interpret, pinning behavior was analyzed using only vehicle, 0.5%, 1.0% and 2.0% conditions (see Figure 2). Analyses of the data on play behavior indicated that the pinning by the ET group was increased in the vehicle condition and reduced to a greater degree by the 2% xylocaine treatment compared to the control groups, indicating a differential effect of the anesthesia. There was a significant group by anesthesia interaction ($F(6,261) = 2.88, p < 0.01$) resulting from the ET group having more pins in the vehicle, 0.5% and 1% conditions but not the 2% condition compared to both control groups (Tukey's HSD, p 's < 0.01).

There was a significant anesthesia by sex interaction ($F(3,261) = 2.73, p < 0.05$) (Figure 3) on pinning. However, analyses of simple main effects of sex did not reveal any significant effects within each sex across the different anesthesia conditions.

There was also a main effect of anesthesia ($F(4,348) = 20.3, p < 0.001$). Bonferroni t-tests indicated that 2% xylocaine reduced pins compared to all other injections (p 's < 0.001) when the data were collapsed across all groups. To test if the effects of anesthesia occurred in the control groups, a repeated measures ANOVA was conducted on the data from just the control groups. There was a significant effect of anesthesia condition ($F(3,195) = 5.67, p < 0.001$), which was the result of a significant linear trend ($F(1,65) = 10.8, p < 0.01$). Bonferroni t-tests (p 's < 0.05) indicated that the 2% xylocaine reduced pins in the control groups to a greater degree than either the vehicle and 0.5% xylocaine injections (but not the 1% xylocaine).

C-Fos IR

One-Way ANOVA on c-Fos IR in the somatosensory cortex indicated a significant main effect of group ($F(2, 43) = 4.6, p < 0.02$). Tukey's *post-hoc* tests indicated that perinatal ethanol exposure resulted in a decrease in c-Fos expression compared to non-treated controls (Tukey's HSD, p 's < 0.05). The observed c-Fos IR was specific to the somatosensory cortex corresponding to the shoulder region (Paxinos and Watson, plates 46-52) (see Figures 4 and 5).

Two-way ANOVA on c-Fos IR in the nucleus accumbens revealed a significant interaction between sex and group ($F(2, 40) = 5.9, p < 0.01$). Further analysis of c-Fos IR in nucleus accumbens of females via analyses of simple main effects indicated a significant effect of group ($F(2, 20) = 3.6, p < 0.05$), which was the result of the c-Fos IR in the ET females being greater than that in the IC females but not the NC females (Tukey's HSD, $p < 0.05$) (see Figures 6 and 7). Analyses of simple main effects also indicated a significant effect of group on c-Fos IR nucleus accumbens in males ($F(2,20) = 4.8, p < 0.05$), which was the result of the c-Fos IR in the ET males being less than that in the NC but not IC males (Tukey's HSD, $p < 0.05$) (see Figures 6 and 7).

Average c-Fos IR cells and standard error of the means (SEMs) for the ET, IC, and NC groups in the dorsal striatum were 59.23 ± 9.59 , 73.87 ± 13.10 , and 80.67 ± 13.43 respectively. C-Fos IR cells and SEMs for the ET, IC and NC groups in the pre-limbic cortex were 156.9 ± 18.10 , 111.57 ± 17.83 , and 179.27 ± 22.42 respectively. One-Way ANOVAs on c-Fos IR in dorsal striatum ($F(2, 43) = .790, p > 0.05$) and pre-limbic cortex ($F(2, 43) = 3.90, p > 0.05$) revealed no significant effects of treatment. The animal numbers were between 15 and 17 for each group for each of the brain regions.

DISCUSSION

Alcohol exposure across all three trimester equivalents increased the frequency of pinning behavior in rats of both sexes. There were no observable effects of perinatal ethanol on the frequency of dorsal contacts. The degradation of somatosensory processing by xylocaine administration into the nape of the neck reduced pinning in all groups; this means that there was no floor effect in the control groups. However, the 2% xylocaine administration caused a much greater reduction in pinning in the ethanol-treated group compared to the control groups without affecting dorsal contacts. Neuronal activation, as measure by c-Fos IR, after one hour of play was reduced in the trunk area of the somatosensory cortex in ethanol-exposed animals of both sexes. In the nucleus accumbens, ethanol-exposed females showed an increase and ethanol-exposed males showed a decrease in c-Fos expression compared to one of the control groups. No significant changes in neuronal activation were observed in the dorsal striatum or the prefrontal cortex.

There are a number of interpretations of the data. The most parsimonious and most consistent with the literature is that alcohol exposure during development results in an increase in somatosensory thresholds in the nape of the neck. Therefore, the ethanol-exposed rat only responds to a nape attack when the other rat is right on top of it and so is restricted to being pinned as the only viable defensive response [53]. This restriction in selection of response during play results in an overall increase in pinning by the pairs of ethanol-exposed rats. The decrease in sensitivity to somatosensory cues was reflected both by the increased sensitivity to somatosensory degradation and the decreased c-Fos IR in the somatosensory cortex in the ethanol-exposed rats in the current study. Prior literature has also indicated that somatosensation and the somatosensory cortex are targets of perinatal ethanol exposure [1, 29,30,39,43,57]. Research from our own lab has also suggested that another form of social behavior, sexual behavior in female rats, may be disrupted in part due to difficulties in processing somatosensory cues [14].

There are a number of alternative explanations. It is possible that the increased pinning in ET rats is not due to somatosensory changes and that there may be changes in other neural structures involved in play that compensate for a change in the somatosensory system that would normally reduce pinning. The brain changes that would negate the lack of activation in the somatosensory cortex could either be directly from alcohol or compensatory to alcohol's effects. A possibility is that the vibrissae input has increased to compensate for cell loss in the somatosensory cortex; however, Rema and Ebner (1999) have shown that the response to test stimuli to the whiskers is reduced by prenatal alcohol exposure even in adulthood [58].

A second alternative explanation is that there may be changes in some of the neural structures involved in the motor and/or motivational aspects of play. The lack of effect of ethanol exposure on dorsal contacts, which is considered to be a measure of motivation, suggests no effects on motivational structures. Furthermore, in another study from our laboratory, there was no differential effect of increasing amounts of isolation (which should enhance social motivation) on ethanol-exposed rats compared to controls on running to gain access to a conspecific, suggesting that the changes in social behavior were not a function of motivation [25]. However, in contrast, the current findings on the nucleus accumbens suggest that there is a sexually dimorphic effect of alcohol exposure on motivation during play behavior. While the current behavioral data do not reflect any sexually dimorphic effects (see below for a more complete discussion of this aspect of the findings), further exploration of the nature of play motivation in alcohol-exposed animals is warranted.

A final alternative interpretation focuses on neural plasticity. Studies have suggested that neural plasticity in both the somatosensory [58] and visual cortices [35] is suppressed by exposure to alcohol during development. It is possible that habituation within a the play period is dependent upon neuroplasticity in the sensory cortices and the impaired neuroplasticity observed in ethanol-exposed animals results in much higher levels of play. However, this study used only a five min play period, which is very short and unlikely to be affected by habituation to a great degree. Nevertheless, tests of play over a longer period of time may be impacted by ethanol-induced deficits in neuroplasticity.

Neuronal activity of the somatosensory cortex, previously shown to be highly activated following play [17,48,63], was significantly decreased in ethanol-treated compared to non-treated control rats, thus indicating an impaired ability to process relevant environmental stimuli (such as dorsal contacts) necessary for social (play) behavior. The decline in c-Fos IR positive cells may reflect a loss of cortical neurons or disorganization in body surface representation in the somatosensory cortex following perinatal ethanol exposure [1,29,30,39] or a change in input to this cortical area. It should be noted that cell loss does not necessarily translate into loss of activation in a neuronal region not does loss of activation in a region imply

loss of cells. Neuronal cell loss can be compensated for by an increase in activation of the remaining cells within the region or alternatively, activation of cells might be severely impaired by changes in connectivity to a region even though there is no cell loss. While there are no studies using stereological cell counts of the somatosensory cortex after alcohol exposure during development, other measures related to cell loss, such as volume of whisker barrels and counts of GAD IR in the somatosensory cortex, suggest that the cell loss is less than 30% and likely around 20% [1,29,30,39]. The current finding of a decline of 35% of c-Fos IR after play in this area suggests that the deficit cannot be explained by simple cell loss and is likely the result of changes in connectivity either within the somatosensory cortex or in its inputs.

Given the importance of the nucleus accumbens in mediating rewarding behavior and the rewarding nature of play [20], the differential expression of the *c-fos* gene suggests the effect of perinatal ethanol exposure on play may involve sex specific changes in motivation. Similarly, Gass et al. also found a sexually dimorphic activation of *c-fos* by a cocaine-paired compartment, although the effect was the reverse of the current findings and consistent with the behavior findings in that study [13]. Thus, developmental ethanol exposure may induce sex specific activity changes in the nucleus accumbens resulting in changes in motivation. However, the current data failed to find any differences in motivation as measured by dorsal contacts. Nevertheless, sexually dimorphic patterns of play and ethanol effects may have been obscured due to experimental design and the effects of ethanol exposure during development on motivation to play warrant further study.

Contrary to previous reports, there was no sex-dependent pattern evident in pins or dorsal contacts in either controls or in the effects of ethanol exposure [37]. Play behavior has been suggested to be sexually dimorphic with males initiating more playful bouts than females (see [54] for a review). Meyer and Riley (1986) reported a sexually dimorphic difference in pinning in controls, which was reversed by prenatal ethanol exposure [37]. However, the current study utilized a short testing period, five minutes, that does not yield reliable dimorphic patterns of play [reviewed in 47, and 69]. Perhaps, the use of a longer testing period in the current study would have allowed a sexually dimorphic pattern to be observed. Additionally, the extent of social isolation in the current study, 7 days, in conjunction with the short test period may have caused a ceiling effect, artificially raising play in both sexes to a maximal level eliminating any dimorphic behavioral patterns [49]. Sexually dimorphic patterns in response tactics during social play have also been reported (for review see [54]). Although we were unable to determine response strategies in the current study, and saw no dimorphic effects on dorsal contacts or pinning, future efforts will be made to identify sex-specific behavioral effects that would be consistent with the ethanol-induced effects on c-Fos IR in the nucleus accumbens.

While the behavioral findings presented here are the result of differences between the ET groups and both control groups, the differences in the findings on c-Fos IR are between the ET group from only one of the control groups. In particular, the difference in c-Fos IR in the somatosensory cortex and in the nucleus accumbens in females is between the ET and IC groups only whereas the difference in c-Fos IR in the nucleus accumbens in males is between the ET and NC groups only. The purpose of the inclusion of both control groups is to control for the stress of the administration procedures of ethanol. When the ET group only differs from one of the control groups, the interpretation of the data is limited by the caveat that the ethanol effect may in part be due to an interaction of ethanol and stress even though the two control groups are not significantly different from each other. Given the prevalence of stress effects during development (see for example, [12 and 19] for a review), it is not surprising that there may be effects of the administration procedures for ethanol.

Extension of the current findings to human infants and children suffering the effects of perinatal ethanol exposure is complicated by the lack of directly comparable measures. However, given

the startlingly high frequency of social deficits evident in exposed human populations [61, 64,68], attempts should be continued to identify comparable measures. Indeed, social play in both humans and rats is believed to be a functional building block for development of adult social behaviors. In rats, play is thought to underlie a variety of cognitive and/or emotional processes including attachment, social organization, and communication that may parallel deficits seen in ethanol-exposed human populations [65,66,68]. Importantly, ethanol-exposed human infants also demonstrate alterations in play [21]. The current data suggest that ethanol exposure during development alters play behavior by alterations in the response to sensory stimulation that is mirrored by a reduction in neuronal activity in the somatosensory cortex after play. When the current data are viewed in the context of previous findings from both our laboratory and others, an emerging theme is that changes in multifaceted behaviors, such as social behavior, may be the result of alterations in the ability to process complex sensory cues appropriately. This theme requires more experimental support but if true, suggests possibilities with respect to treatment of deficits in FASD.

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Figure 1. Frequency of dorsal contacts

Ethanol treatment was without effect on dorsal contacts at all doses of xylocaine. Data are collapsed across sex. Error bars represent standard errors of the mean (SEM).

Figure 2. Frequency of Pinning

Ethanol treatment induced an elevation in pinning at 0, 0.5% and 1% xylocaine. While 2% xylocaine significantly reduced pinning in all groups, there was a greater effect of 2 % xylocaine in the ET group compared to both control groups. Data are collapsed across sex. Error bars represent SEM and stars indicate significantly different from both control groups at that xylocaine dose.

Figure 3. Effects of anesthesia on pinning by the two sexes

There was a significant interaction of anesthesia condition and sex but further analyses failed to reveal any specific effects within each sex. Data are collapsed across group. Error bars represent SEM.

Primary somatosensory cortex

Figure 4. c-Fos IR in the primary somatosensory cortex

C-Fos immunoreactivity in the somatosensory cortex of ET (n = 15), NC (n = 15), and IC (n = 16) animals (SH1, plates 48-52 [38]). Data are collapsed across sex. The star indicates a significant difference between ET group and the NC control. Error bars represent SEM.

Figure 5. Representative photomicrographs of c-Fos IR in the somatosensory cortex
c-Fos IR as seen in an NC (A), IC (B), and ET (C) animals are shown. Sections represent primary somatosensory cortex, shoulder region, corresponding to plates 48-52 [38].

Figure 6. c-Fos IR in the nucleus accumbens

A sexually dimorphic effect of ethanol on c-Fos IR in the nucleus accumbens (NAC, plates 16-18 [38]) was seen. ET females had significantly more c-Fos positive cells compared to IC females. ET males had significantly fewer c-Fos positive cells than NC males. Stars indicate a significant difference from one of the control groups. Error bars represent SEM. The subject numbers are 9, 7, and 7 in ET, IC and NC females respectively and 7, 7, and 9 in ET, IC and NC males respectively.

Figure 7. Representative Photomicrographs of c-Fos IR in the nucleus accumbens
C-Fos IR as seen in female NC (A), IC (C), and ET (E) animals and male NC (B), IC (D), and ET (F) animals.

Table 1Average dam weights ($\pm$ SEMs) and subject numbers

Groups	N	Weight on GD 1	Weight on GD 5	Weight on GD 10	Weight on GD 15	Weight on GD 20
ET	20	263.5 $\pm$ 2.9	263.3 $\pm$ 3.4	275.2 $\pm$ 3.7	294.6 $\pm$ 4.6	333.5 $\pm$ 4.9
IC	17	267.9 $\pm$ 4.0	264.7 $\pm$ 5.7	275.2 $\pm$ 4.9	292.0 $\pm$ 5.5	329.4 $\pm$ 7.0
NC	19	260.4 $\pm$ 6.0	272.3 $\pm$ 5.1	287.9 $\pm$ 4.9	309.1 $\pm$ 3.4	341.6 $\pm$ 5.0

Table 2

Average pup weights ($\pm$ SEMs) and subject numbers

Group	Sex	Number	Weight on PD 2 (g)	Weight on PD 10 (g)	Weight on PD 21 (g)	Weight on PD 30 (g)
ET	females	28	6.2 $\pm$ 0.1	17.4 $\pm$ 0.7	42.6 $\pm$ 1.4	78.8 $\pm$ 3.0
ET	male	26	6.7 $\pm$ 0.2	18.3 $\pm$ 0.6	45.1 $\pm$ 1.8	87.8 $\pm$ 2.6
IC	females	30	6.6 $\pm$ 0.2	18.8 $\pm$ 0.5	42.7 $\pm$ 1.3	85.3 $\pm$ 2.0
IC	males	32	7.1 $\pm$ 0.2	19.9 $\pm$ 1.4	46.3 $\pm$ 1.2	95.9 $\pm$ 2.0
NC	females	30	7.0 $\pm$ 0.1	19.1 $\pm$ 0.5	44.8 $\pm$ 1.2	81.9 $\pm$ 2.3
NC	males	40	7.6 $\pm$ 0.1	21.0 $\pm$ 0.3	49.6 $\pm$ 1.0	95.8 $\pm$ 2.1