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The effects of neonatal ethanol and/or cocaine exposure on isolation-induced ultrasonic vocalizations

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Abstract

Isolation-induced ultrasonic vocalizations (USVs) are emitted by young rat pups when isolated from their dam and conspecifics. These USVs play an important role in maternal/offspring interactions, and have been used as an indicator of response to stress and isolation. This study examined the effects of neonatal ethanol and/or cocaine exposure on USVs in neonatal rats. The neonatal exposure paradigm serves as a model for the "human third trimester of pregnancy" in terms of CNS development. There were five treatment groups including an artificially reared (AR) ethanol-exposed group (6 g/kg/day), an AR cocaine-exposed group (60 mg/kg/day), an AR ethanol- and cocaine-exposed group (6 g/kg/day + 60 mg/kg/day), an AR isocaloric control, and a normally reared control. Both groups that received ethanol took longer to vocalize, and displayed fewer vocalizations than non-ethanol-exposed pups when tested on clean bedding (Experiment 1) or on chips from the nest of a lactating dam (Experiment 2). These results suggest that neonatal ethanol exposure alters the pup's immediate response to isolation. This could have direct effects on maternal/infant interactions, and might help explain some of the long-term effects of ethanol exposure on social behaviors.

Keywords: Third trimester drug effects; Polydrug exposure; Social behaviors; Neonatal ethanol exposure; Neonatal cocaine exposure; Ultrasonic vocalizations

1. Introduction

When neonatal rat pups are isolated from their dam or conspecifics they emit ultrasonic vocalizations (USVs). One of the primary functions of these USVs is to elicit maternal attention. USVs evoke pup retrieval and a variety of pup-directed behaviors including pup grooming as well as suppressing or decreasing maternal biting and cannibalism [3,9,10,18,58,71]. USVs also play a role in lactation with their occurrence at the beginning of a nursing bout resulting in shortened latencies to the first maternal oxytocin pulse [19]. These USVs are primarily temperature dependent during the first week of life [2,3,60], with cooler temperatures resulting in higher rates of USVs. In fact, it has been suggested that the USVs produced by neonates in cold environments could contribute to their attempt to thermoregulate by displaying altered respiratory patterns characterized by drawn-out expiratory durations referred to as laryngeal braking [8]. USVs are not simply a temperature-induced phenomenon, however. Pups will reduce their frequency of isolation-induced USVs by the presence of a single littermate or anesthetized dam cooled to room temperature or below [12,29,69]. During the second postnatal week, it is the loss of physical contact with the dam or conspecifics that is one of the most salient cues for eliciting USVs [30,31,61]. Pups during the second week of life are also highly responsive to environmental odors with familiar maternal odors or potentially threatening odors such as an unfamiliar anesthetized male decreasing USV emissions [61,72] and exposure to clean bedding increasing vocalizations [61].

Pharmacological manipulations of a variety of neurotransmitters or neurosteroids can alter the frequency and occurrence of isolation-induced USVs [11,28,36,39,79,86,87,89]. Anxiolytic drugs reduce the frequency of USVs, while anxiogenic drugs increase them (see Refs. [34,35]). Consequently, isolation-induced USVs have been used as an early indicator of response to stress. Acute administration of either ethanol [24] or cocaine [38] has also been shown to suppress USVs.

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Of particular interest for the current study, USVs can also be altered by prenatal manipulations including nitrogen dioxide [21] or stress [84]. Prenatal ethanol exposure may also reduce isolation-induced USVs [40], although this has not been uniformly reported [90]. With the role that USVs play in eliciting maternal attention, there are important social and nutritional implications if rat pups do not produce these vocalizations.

In this study, we assessed the effects of neonatal ethanol and/or cocaine exposure on isolation-induced USVs in neonatal rats. While the majority of animal models developed to study the effects of prenatal drug exposure have focused on the effects of a single drug, this rarely occurs in clinical populations. Of those that use drugs during pregnancy, polydrug use is more common. Among women that use cocaine during pregnancy, ethanol is also frequently consumed [23,37,70]. Therefore, our rodent model was developed to look at the potential interactions between ethanol and cocaine during development. Drugs were administered to the neonatal rat to model the "third trimester" of human pregnancy, in terms of brain development. In humans, this developmental stage is characterized by rapid neuronal growth and proliferation, and thus may be a particularly sensitive period for drug-related effects on the CNS. In rats, this "brain growth spurt" extends into the early postnatal period [7,22].

For the current study, pups exposed to ethanol or cocaine or the combination during the first 2 weeks of life were isolated from their dam and conspecifics and placed over either unscented hardwood chips (Experiment 1) or wood chips from the nest of a lactating dam (Experiment 2) and vocalizations were recorded. Because pups tend to display reduced vocalizations when familiar maternal or conspecific odors are present, it was hypothesized that an isolated pup tested in the presence of the odor from a lactating dam would display lower levels of USVs in contrast with pups tested over clean chips that would display an augmentation of USVs relative to those tested over chips from a maternal nest [61].

2. Materials and methods

2.1. Mating procedure

Parent animals were Sprague–Dawley rats obtained from Harlan Labs (Indianapolis, IN) that were bred in the animal facility at the University of Kentucky. Pregnant females were individually housed in plastic cages in a temperature-controlled nursery that was maintained on a 12L:12D cycle. As parturition approached, females were checked twice daily for birth. The day of birth was postnatal day 0. Twenty-four hours following parturition, litters were culled to 10 pups, maintaining 5 males and 5 females when possible.

2.2. Artificial rearing procedure

On postnatal day (PND) 4, one male and one female pup from each litter were randomly assigned to one of five treatment groups: artificially reared (AR) receiving 6 g/kg/day ethanol, AR receiving 60 mg/kg/day cocaine hydrochloride, AR receiving both 6 g/kg/day ethanol and 60 mg/kg/day cocaine hydrochloride, an AR control (stock), or a sham surgery control (sham). Each pup was lightly anesthetized with halothane (50% halothane/50% compressed air) and an intragastric cannula made of polyethylene tubing (Clay Adams PE-10) was implanted into the stomach. This surgical procedure takes approximately 1–2 min to complete, and has been described in detail in previous reports (e.g., Refs. [5,67]). The sham controls underwent a similar surgical procedure; however, the intragastric cannula was not implanted.

Upon recovery from anesthesia, the shams were returned to their home cage to be reared by lactating dams. The AR pups were individually housed in Styrofoam cups that were situated in a second Styrofoam cup with sand as ballast and that floated in a heated and aerated water bath. The water bath was maintained at 48°C on PND 4–5 and reduced by 2°C every 2 days thereafter, resulting in a final temperature of 42°C on PND 11. The cups were filled with approximately 5 cm of wood chips and a piece of artificial fur that lined one side of the cup to help reduce any behavioral depression associated with artificial rearing [78].

The feeding cannula was attached to Tygon tubing that was connected to a syringe containing a formula designed to mimic rat milk [83]. The AR pups were fed the milk solution for 20 min every 2 h with the aid of a multi-syringe infusion pump and timer. Ethanol and cocaine were added to the milk solution for 4 of the 12 daily feeding periods to mimic a "binge" model of drug exposure. Maltose–dextran was added to the diet of the 60 mg/kg/day cocaine AR group and the AR stock control to make these groups isocaloric with the ethanol-exposed groups. During the remaining feeding periods, all drug-exposed subjects received the stock milk solution.

Each day, the intragastric cannulas were flushed with distilled H_2O and the AR pups were weighed. Fresh chips were added to the cup as needed, and the chips were completely changed in the cup on PND 7. The amount of milk administered daily was equal to 30% of the average body weight of all of the AR pups for that day. The bladders of the pups were voided three times daily by genital stimulation of the anogenital region with a cotton swab. Pups were maintained in this manner from PND 4–10. On PND 10, all AR pups were fed the stock milk solution to allow the pups a 24-h drug-free period to avoid any possible withdrawal effects before being returned to their lactating dam. It should be noted that there was no obvious evidence of withdrawal among any of the treatment groups (wet dog shakes, excessive grooming, etc). The sham control pups were also handled and weighed daily.

On PND 11, the intragastric cannulas of the AR pups were clipped close to the abdominal wall and the AR pups and sham controls were earmarked for later identification. All pups (AR and sham control) were bathed in a warm slurry of maternal feces and water and then returned to a lactating dam in a cage with clean bedding. This procedure virtually eliminates maternal rejection or pup mortality.

2.3. Postnatal behavioral testing

USVs were recorded with the aid of an ultrasonic bat detector (Ultra Sound Advice) set at 40 kHz. On PND 13, each pup was briefly removed from its litter and marked with a permanent marker to color-identify the subject, which ensured that the experimenter recording vocalizations was blind to treatment condition. Testing was conducted on PND 14 for both Experiments 1 and 2. For each experiment, the dam was removed from the litter, placed in a holding cage, and returned to the cage rack. The pups were kept in their home cage that was placed on a heating pad until testing.

Each pup was tested in isolation at room temperature. The pup was removed from its home cage and placed in a glass test compartment (21 cm long × 11 cm wide) that contained 250 ml of clean unscented wood chips (Experiment 1) or chips taken from the nest of an unfamiliar lactating dam (Experiment 2). The same chips were used for all subjects on a given test day, and for Experiment 2, the chips were taken from the nest of a litter that was approximately the same age (± 1 day). The purpose of using these two odors was to examine whether neonatal ethanol and/or cocaine exposure altered the USV response to these environmental cues. The microphone was placed 20 cm above the center of the test chamber. The experimenter wore earphones connected to the bat detector and recorded the number of vocalizations using a real-time event recorder. The length of the test session for each experiment was 5 min. At the conclusion of the test session, body weights were recorded. A range of 10–12 subjects/sex and neonatal treatment group were included in each experiment and pups were tested in either Experiment 1 or 2.

2.4. Data analysis

The number of vocalizations/minute and the latency to vocalize were measured using a 2 × 2 × 2 factorial design with ethanol, cocaine, and sex as between-group factors. This statistical approach was chosen because these studies were designed to study the potential interaction of ethanol and cocaine, and this analysis directly addresses this question. For these analyses, the stock and sham groups were combined into a single control group as a separate overall ANOVA did not reveal any significant differences between these control groups. Where examination across time was required, the analyses included 1-min blocks as the repeated measure (five 1-min blocks). Because there was no main effect of sex and it did not interact with any other variable in either experiment, the data were collapsed across sex, resulting in 2 × 2 (× 5 for repeated measures) factorial analyses.

3. Results

3.1. Body weights during artificial rearing

Pup body weights during the neonatal drug administration period are presented in Table 1. Because the experiments were conducted at different time points, the data from each experiment was analyzed separately. In Experiment 1, there was a significant neonatal treatment × day interaction, $F(4,105) = 70.252$, $p < 0.001$. Subsequent simple main effect analyses revealed significant treatment effects ($ps < 0.05$) on all days except PND 4. Pairwise comparisons for these significant main effects were subse-

Table 1
Body weights during artificial rearing (in g) ± SEM

Treatment	PND 4	PND 5	PND 6	PND 7	PND 8	PND 9	PND 10	PND 11
Experiment 1								
Sham control	10.6 ± 0.2	12.5 ± 0.2	14.6 ± 0.3	16.5 ± 0.3	19.1 ± 0.3	21.1 ± 0.3	23.8 ± 0.3	26.4 ± 0.4
Stock control	11.4 ± 0.2	13.5 ± 0.1[a]	15.0 ± 0.2	16.3 ± 0.2	18.1 ± 0.2[b,c]	19.6 ± 0.2[a,b,c]	20.6 ± 0.3[a,b,c]	22.3 ± 0.5[a]
Ethanol	11.5 ± 0.2	13.7 ± 0.2[a]	15.4 ± 0.2[a]	16.6 ± 0.2[b]	18.3 ± 0.3[b,c]	19.6 ± 0.3[a,c]	21.1 ± 0.2[a,b,c]	22.2 ± 0.3[a]
Cocaine	11.3 ± 0.2	13.2 ± 0.2	14.6 ± 0.2	15.6 ± 0.2	16.6 ± 0.2[a]	18.1 ± 0.3[a,b]	19.2 ± 0.3[a]	21.1 ± 0.4[a]
EtOH/cocaine	10.8 ± 0.5	13.1 ± 0.2	14.7 ± 0.2	15.9 ± 0.2	16.9 ± 0.3[a]	18.2 ± 0.3[a]	18.9 ± 0.4[a]	20.8 ± 0.4[a]
Experiment 2								
Sham control	10.3 ± .0.3	11.5 ± 0.3	13.0 ± 0.4	14.8 ± 0.4	16.3 ± 0.4	18.3 ± 0.5	20.5 ± 0.5	23.1 ± 0.5
Stock control	11.1 ± 0.2	12.8 ± 0.2	14.5 ± 0.3	15.6 ± 0.3	17.0 ± 0.3	18.9 ± 0.3	20.6 ± 0.4	21.9 ± 0.5
Ethanol	10.7 ± 0.2	12.2 ± 0.3	13.9 ± 0.3	15.2 ± 0.3	16.5 ± 0.3	18.4 ± 0.3	20.0 ± 0.3	20.9 ± 0.5
Cocaine	10.6 ± 0.2	12.1 ± 0.2	13.5 ± 0.3	14.5 ± 0.2	15.7 ± 0.3	17.1 ± 0.4	18.6 ± 0.4	20.7 ± 0.5
EtOH/cocaine	11.2 ± 0.2	12.7 ± 0.2	14.2 ± 0.2	15.3 ± 0.2	16.4 ± 0.2	17.7 ± 0.3	18.7 ± 0.3	20.2 ± 0.5

[a] Significantly differs from sham control.
[b] Differs from cocaine group.
[c] Differs from EtOH/cocaine group.

Table 2
PND 14 body weights (in g) ± SEM

	Experiment 1	Experiment 2
Sham control	36.2 ± 1.2[a]	31.9 ± 0.8[a]
Stock control	28.9 ± 0.7	26.6 ± 0.7
Ethanol	29.9 ± 0.6	25.0 ± 0.7
Cocaine	28.9 ± 0.6	24.7 ± 0.5
Ethanol + cocaine	28.0 ± 0.7	26.1 ± 0.5

[a] Significantly different from all other treatment groups.

quently conducted using a Bonferroni correction for multiple comparisons ($p = 0.006$). The complex pattern of group differences is shown in the upper half of Table 1. Briefly, the increments in body weight appeared lower for the cocaine and ethanol/cocaine AR groups, relative to the other treatment groups. Furthermore, on PND 11, all of the AR treatment groups weighed less than the sham controls. In contrast, there were no effects of neonatal treatment on body weight in Experiment 2.

Body weights taken at the conclusion of USV testing on PND 14 are presented in Table 2. The results were similar for both experiments. The overall ANOVAs yielded a significant main effect of neonatal treatment, $F(4,110) = 30.74$, for Experiment 1 and $F(4,93) = 19.41$, $ps < 0.001$, for Experiment 2. Subsequent Newman–Keuls's analyses revealed that all of the AR groups weighed less than the sham controls ($ps < 0.01$). There were no differences in body weight across the AR groups.

3.2. Experiment 1

The latency until the first vocalization over unscented wood chips is shown in Fig. 1. The 2 × 2 ANOVA revealed a marginal main effect of ethanol, $F(1,116) = 3.87$, $p = 0.051$. While only marginally significant, it suggested that the ethanol- and ethanol/cocaine-exposed groups took longer to vocalize than those not exposed to ethanol (i.e., the cocaine-exposed group and the control group). Further support for this altered pattern of USVs by the ethanol- and ethanol/cocaine-exposed offspring was apparent by their frequency of vocalizations across the test session. Although the number of vocalizations increased across trials for all treatment groups, the ethanol- and ethanol/cocaine-exposed offspring displayed fewer vocalizations than offspring not exposed to ethanol as shown in Fig. 2. The 2 × 2 × 5 repeated-measures ANOVA on the number of vocalizations across each 1-min block of the test session revealed a main effect of ethanol, $F(1,116) = 7.89$, $p < 0.01$ and a main effect of trial, $F(4,464) = 60.84$, $p < 0.001$. There were no other significant effects or interactions.

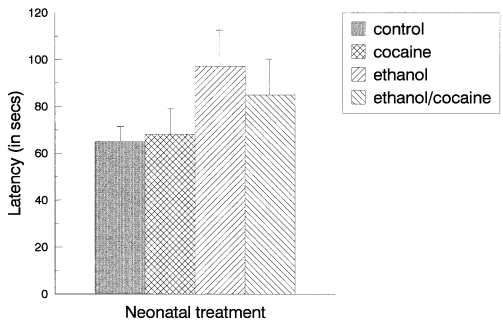

Fig. 1. Average latency (+SEM) to display USVs when tested over unscented chips (Experiment 1), as a function of neonatal treatment (control, 6 g/kg/day ethanol (EtOH), 60 mg/kg/day cocaine (COC), 6 g/kg/day EtOH + 60 mg/kg/day COC in combination, respectively). Since there was no effect of gender, the data are collapsed across this variable.

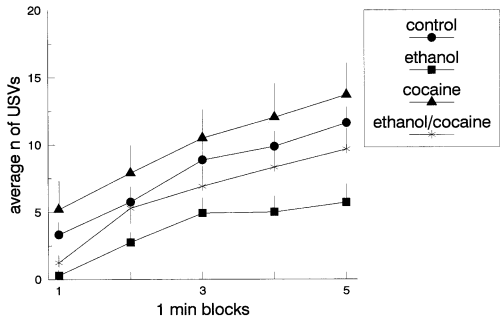

Fig. 2. Average number of USVs (+SEM) when tested over unscented chips (Experiment 1) for each 1-min block as a function of neonatal treatment (control, 6 g/kg/day ethanol (EtOH), 60 mg/kg/day cocaine (COC), 6 g/kg/day EtOH + 60 mg/kg/day COC in combination, respectively). Since there was no effect of gender, the data are collapsed across this variable.

3.3. Experiment 2

The latency for the first vocalization when placed over wood chips from the nest of a lactating dam was examined using a 2 × 2 ANOVA. This ANOVA revealed a significant main effect of ethanol, $F(1,97) = 4.45$, $p < 0.05$. As in Experiment 1, both ethanol-exposed groups (ethanol and ethanol/cocaine) took longer to emit their first USV (see Fig. 3) and displayed fewer vocalizations throughout the test

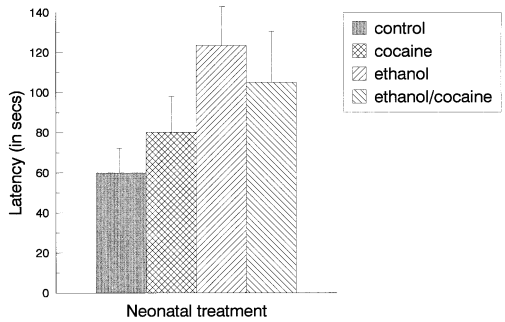

Fig. 3. Average latency (+SEM) to display USVs when tested over chips from the cage of a lactating dam (Experiment 2) as a function of neonatal treatment (control, 6 g/kg/day ethanol (EtOH), 60 mg/kg/day cocaine (COC), 6 g/kg/day EtOH + 60 mg/kg/day COC in combination, respectively). Since there was no effect of gender, the data are collapsed across this variable.

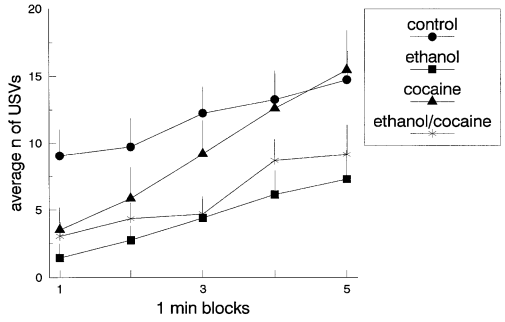

Fig. 4. Average number of USVs (+ SEM) when tested over chips from the cage of a lactating dam (Experiment 2) for each 1-min block as a function of neonatal treatment (control, 6 g/kg/day ethanol (EtOH), 60 mg/kg/day cocaine (COC), 6 g/kg/day EtOH + 60 mg/kg/day COC in combination, respectively). Since there was no effect of gender, the data are collapsed across this variable.

session relative to groups that did not receive ethanol (see Fig. 4). The 2 × 2 × 5 repeated-measures ANOVA on the number of vocalizations over the 1-min blocks revealed a main effect of ethanol, $F(1,97) = 8.28$, $p < 0.05$. Again, the number of vocalizations increased across time for all treatment groups as indicated by a main effect of trial, $F(4,388) = 29.91$, $p < 0.001$.

Although Experiments 1 and 2 were conducted independently, our original hypothesis was that isolating pups over unscented wood chips or over chips from the nest of a lactating dam would make a difference in latency and rate of USVs with USVs being emitted earlier and with higher frequency if pups were tested on unscented bedding [61]. Furthermore, it was possible that this effect could be treatment group-dependent. To directly address this question, the data from both experiments were combined in a single analysis to look at the test chamber manipulation (unscented or nest chips). There were no differences in either the latency to vocalize or in the number of USVs across time as a function of this environmental manipulation nor did it interact with neonatal treatment or sex ($p > 0.25$).

4. Discussion

Rat pups neonatally exposed to ethanol or ethanol and cocaine in combination showed reductions in isolation-induced USVs. These pups took longer to vocalize when isolated, and they also vocalized less throughout the test session relative to controls or the cocaine-exposed offspring. These results were similar whether the pups were tested over unscented chips or chips from the cage of a lactating dam. These findings are the first to show that neonatal ethanol exposure reduces isolation-induced USVs and these results support similar findings following prenatal ethanol exposure [40], although it should be noted that not all studies have reported suppression of isolation induced vocalizations following prenatal ethanol exposure [90].

The data from these two experiments suggest that the suppression of USVs were most likely attributable to the effects of ethanol. The ethanol/cocaine- and ethanol-exposed groups were similar in their USV response to isolation, suggesting no synergistic effects of ethanol and cocaine on this behavioral response. These findings also suggested that neonatal cocaine exposure had little effect, either on its own or in combination with ethanol. This absence of a cocaine-related effect was not surprising, and supports previous data suggesting that neonatal cocaine exposure does not alter isolation-induced USVs [51]. It should be noted, however, that previous studies from our laboratory have shown that exposure to the ethanol/cocaine combination can have more adverse effects than exposure to either drug alone in certain learning paradigms [4,68].

The distinction between Experiments 1 and 2 was differences in the odor cues in the environment with exposure to unscented clean wood chips in Experiment 1 and bedding from the nest of a nonfamiliar lactating dam in Experiment 2. It was expected that exposure to unscented chips would result in an augmentation of USVs at least among control pups [48]. However, this was not observed for any of the treatment groups. It is possible that repeated exposure to unscented chips (both in the AR environment and the frequent bedding changes in the home cage) resulted in habituation to the odor of unscented chips. This might explain why there were no differences in the rate or onset of USVs across Experiments 1 and 2. Another potentially interesting question was based on previous data that suggested that pups at younger ages (up to PND 7) vocalized more when exposed to bedding from the nest of a nonfamiliar lactating dam than when tested with bedding from their own home cage or unscented chips [16]. While this increased rate of vocalization decreases as the pup matures [16], prenatal and neonatal ethanol exposure has been shown to result in a variety of developmental delays [52], and so it was conceivable that they would still be responsive to the odor of a nonfamiliar dam. Again, this was not the case. Still, the significant conclusion remains that the suppression of USVs displayed by ethanol-exposed offspring was present whether the ethanol-exposed pups were exposed to odors from a lactating dam or unscented chips.

There were body weight differences during the AR rearing procedure between Experiments 1 and 2. It is not unusual that AR pups gain weight more slowly during the AR period relative to sham surgery controls. This pattern was observed in Experiment 1 and, additionally, it appeared that the groups that received cocaine (either the ethanol/cocaine or the cocaine alone) gained weight more slowly than the other AR groups. This cocaine effect has not previously been reported in our laboratory and may simply be variation or sampling error because it was not observed in Experiment 2. Because this body weight difference in Experiment 1 was linked to cocaine, rather than ethanol, it is unlikely that it

contributed to the suppression in USVs displayed by the ethanol-exposed pups. Furthermore, the body weight differences at the time of testing (i.e., an AR effect) probably did not contribute to the augmentation of USVs by ethanol-exposed neonates because the AR pups that did not receive ethanol showed normal USV responses to isolation.

Artificial rearing did not alter the normal USV response to isolation as indicated by similar levels of USVs between the AR and sham control groups. This was surprising, because the AR pups had experienced extended periods of isolation during the AR procedure. The isolation associated with AR is one of the major drawbacks of this drug administration method, and we were concerned that the AR groups might react differently to the isolation test simply because they had habituated to isolation. This was not the case, and these results strongly suggest that contact with the dam and/or conspecifics (and removal) was as salient for the AR pups as it was for the normally reared sham surgery pups. This relatively normal USV response following prolonged isolation has been previously reported by Hofer et al. [32]. Thus, there appears to be an inherent biological predisposition to vocalize when isolated after contact with the dam and litter regardless of early experiences.

It is possible that suppression of USVs displayed by AR ethanol-exposed pups could be due to differences in their response to isolation and the AR procedure. For example, there may be an interaction between ethanol exposure and AR. It is unlikely that an ethanol AR interaction alone can explain the results from this study, however, because recent pilot data collected in our laboratory indicates that pups that receive ethanol by oral intubation (and are reared normally by their dams) also show a suppression in isolation-induced USVs. Thus, this effect appears to be due to ethanol exposure rather than the rearing procedure.

Because USVs are considered an early stress response, the results from this study may be explained by an enhanced stress response to the isolation by the ethanol-exposed offspring. Reduced vocalizations are an indicator of increased stress [34], although the converse has also been argued [31]. While this may sound contradictory, it makes ethological sense that during periods of high stress (i.e., presence of a predator) or low stress (presence of nesting material from the home cage), vocalizations should only occur at a low level. Considerable work has been done to try and determine the role of the hypothalamic–pituitary–adrenal (HPA) axis in USVs. The role that this axis plays in isolation-induced USVs is complex. USVs do not appear to be directly correlated with corticosterone response [45], and contrary to predictions, early studies suggested that intracerebroventricular injections of CRF reduced USV calls [34]. More recent studies, however, suggest that centrally administered CRF has a biphasic effect on USVs with low doses initiating USVs but higher doses diminishing them [28]. These endocrinological findings complement the ethological explanation described above. Pre- and neonatal ethanol exposure has been associated with augmented response to stressors as measured by behavioral and endocrinological endpoints [1,42,44,76,77,81]. Thus, it is conceivable that the reduction in USVs displayed by the ethanol-exposed pups could be due to an elevated reaction to the isolation test. Further studies looking at endocrinological measures may help clarify why there is a reduction in USVs among the ethanol-exposed pups.

The HPA and its associated hormones have also been implicated by Takahashi and others in the development of behavioral inhibition (e.g., Ref. [74]). For example, adrenalectomized neonates do not suppress vocalizations when confronted with threatening stimuli (unfamiliar male rats) [72,73]. These authors further suggest that manipulations that reduce the release of hypothalamic CRF reduce inhibitory control and thus, should increase USVs [74]. On numerous behavioral indices, prenatal and neonatal ethanol exposure are associated with deficits in inhibitory control [52] and hypothalamic CRF levels are reduced in neonatal males following prenatal ethanol exposure [63]. However, this explanation appears unlikely to explain the findings from the current study because USVs are reduced rather than increased in the ethanol-exposed groups.

Because USVs play such an important role in maternal/infant communication, one would expect changes in maternal behavior directed toward these ethanol-exposed offspring. There have been previous reports that ethanol-exposed pups are not retrieved as readily by dams as non-treated pups [25]. One possible explanation for these findings is that ethanol-exposed pups do not produce the necessary USV cues that elicit maternal attention and retrieval.

Appropriate levels of maternal behavior and interaction with pups are critical for normal development. For example, dams spend more time grooming the anogenital region of male relative to female pups, and this has long-term implications in the expression of male copulatory behavior [54,55]. Pup grooming and licking also has long-term effects on the development of the HPA axis and response to stress in adult offspring [47]. It is interesting to note that both copulatory behavior [80] and stress responses are altered following prenatal and/or neonatal ethanol exposure [41,59,82]. Consequently, some of these long-term behavioral effects associated with early ethanol exposure could be mediated, at least in part, by altered maternal behaviors directed toward these pups due to the pups' failure to display normal maternal eliciting cues.

As stated previously, pharmacological manipulations of a number of neurotransmitters can alter the rate and occurrence of USVs. Manipulations of noradrenergic, GABA, glutamate, or neurosteroid levels can alter USVs (e.g., Refs. [24,35,36,39,79,86,89]). It is interesting that many of these neurotransmitter systems are also affected by either prenatal or neonatal ethanol exposure (e.g., Refs. [20,56,66,90]). It is possible that alterations in one or more of these transmitter systems contribute to the ethanol-related effects reported in this study.

Prenatal and/or neonatal ethanol exposure also alters a variety of social behaviors in rodents including aggression [65,88], play [53], maternal behavior [6,27,85], and social communication [43]. In virtually all of these social behaviors, USVs play a critical role (e.g., Refs. [26,46,50,62,75]). If the suppressive effects of neonatal ethanol exposure on USVs persist, these deficits in communication could contribute to many of the problems in social behaviors described above.

These findings also have potential clinical implications. Environmental factors including maternal/infant interactions are particularly important for the outcome in "at risk" infants [14]. For example, positive postnatal environment and parent/infant interactions can result in improved outcome in "at risk" infants on measures of cognitive function and social development [13,17,33,49,64]. In contrast, abnormal or impaired maternal interactions with infants can have persistent negative effects on cognitive function [57]. Coles and Platzman [15] have suggested that the neonatal/postnatal environment can also play an important role for the outcome of children exposed to ethanol and/or other drugs in utero. If drug-exposed infants do not respond or display the normal cues for eliciting maternal/parental contact or interactions, it could have a significant impact on the outcome for these offspring.

In conclusion, USVs play an important role in communication in rats in a variety of settings. Specific to this study, isolation did not produce the normal USV response by ethanol-exposed pups. This reduction in USVs may contribute to altered interactions between ethanol-exposed pups and their dam, which could help explain many of the effects reported following early ethanol exposure. Further work is needed to determine whether neonatal ethanol exposure also reduces the ability of environmental cues to elicit USVs in adults and/or other settings.

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