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Ethanol exposure during development reduces resident aggression and testosterone in rats

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Abstract

Ethanol exposure during development has been shown to alter social behaviors in people, but the range of deficits is not clear. Using an animal model of Fetal Alcohol Spectrum Disorders, inter-male aggression and testosterone levels were examined in adult rats. Rats were exposed to ethanol during the entire prenatal period and from postnatal day 2 through 10. Ethanol was administered via intragastric intubation. Two other groups consisted of a nontreated control and an intubated control group that was exposed to the administration procedures but not ethanol. Both offensive and defensive aggression were examined in experimental residents and intruders under three different housing conditions for the resident males: (1) with another male, (2) with a pregnant female, and (3) with a female and litter fathered by the experimental animal. When housed with a female and litter, ethanol-exposed rats displayed reduced offensive aggression compared to control groups under the same condition. Defensive aggression in the non-experimental intruders was reduced in this same condition. There were no differences in duration of non-aggressive social behaviors among the groups in any of the housing conditions. Testosterone levels were reduced in ethanol-exposed rats compared to controls. In summary, ethanol exposure over the combined prenatal and postnatal periods reduces aggressive behavior in a condition where aggressive behavior is normally seen. This reduction may be related to lower testosterone levels.

Keywords

Fetal alcohol syndrome; Aggression; Resident–intruder paradigm; Social behavior; Prenatal alcohol

Fetal Alcohol Spectrum Disorders (FASD) describes those individuals who manifest mild to severe disturbances of physical, behavioral, emotional, and/or social functioning due to in utero ethanol exposure. FASD individuals are found to commit high rate of crimes against others, engage in inappropriate sexual behavior, and are described as having a failure to consider the consequences of their actions [1,2]. Even though FASD individuals show high rates of crime, no direct measures of aggressive behaviors or testosterone in FAS individuals have been conducted.

Animal models of FAS have examined the effect of different time periods of ethanol exposure on inter-male aggression. Ethanol exposure throughout the entire gestation period

in mice results in an increase in aggression [3-6]; ethanol exposure from gestation day (GD) 6 to 19 increases offensive aggression in rats [7]. Ethanol exposure during the last four days of pregnancy and from postnatal day (PD) 1 to PD 4 increases aggression, while ethanol exposure confined to the period from PD 1 to 4 did not affect aggressive behavior in mice [8]. However, another study found that ethanol exposure from birth until PD 14 decreases aggression in mice [6]. These studies suggest that prenatal ethanol exposure may increase aggression, while postnatal ethanol exposure may decrease aggression. However, many of the early studies used ethanol administration procedures that were confounded by nutritional effects [4-6,8] or resulted in minimal transfer of ethanol to the pup [6]. The effect of ethanol exposure during the entire gestation period and during early postnatal development (i.e. a period equivalent to all the trimesters in the human) on aggression has not been previously examined.

Prenatal ethanol exposure produces a feminizing effect on male sexual behavior, which is believed to result from decreased testosterone levels [9]. Ethanol exposure from GD 12 to PD 10 decreases testosterone in male rats on PD 55 and PD 110 [10]. Ethanol exposure from GD 10 to 19 augments testosterone levels in male rats on GD 18 and 19 [11] and suppresses it during the early postnatal period [12]. Low levels of testosterone during development and/or during adulthood have been shown to result in low levels of offensive inter-male aggression in rats and mice [13-16]. Thus, the ethanol-induced suppression of testosterone during both the early postnatal period and adulthood suggests that a decrease in inter-male aggression in adulthood should be observed in ethanol-exposed rats [13,16,17].

In the present study, rats were exposed to ethanol during a period that is equivalent to the three trimesters of human prenatal development [18]. This exposure period consists of GD 1 through 22 and PD 2 through 10. This exposure period reflects the drinking pattern of a mother likely to produce an offspring with FASD [19,20]; it has been shown that if a woman stops drinking prior to her third trimester (equivalent to the early postnatal period in the rat), the cognitive effects of the ethanol exposure on the offspring are ameliorated [19]. Furthermore, ethanol given during all three trimester equivalents in rats can have behavioral and neural effects that are not easily predicted from the effects of shorter periods of ethanol exposure [21,22], making it important to use the extended exposure period in order to enhance generalizability to the human condition. Because changes in testosterone can result in changes in aggressive behavior [13,15], it was hypothesized that ethanol exposure during the three-trimester equivalent period would decrease both offensive aggression and testosterone levels in male Long–Evans rats tested as residents in the resident/intruder paradigm [23]. Three different housing conditions (which included housing with a male, with a pregnant female, and with a female and pups) for the rats were used because the different housing conditions result in different levels of aggression [15] thus increasing the possibility of detecting differences in aggression across groups and giving contextual specificity for any changes in aggression. In addition, it was hypothesized that the decrease in offensive aggression in the ethanol-exposed animals should also be reflected in a decrease in defensive behavior in the intruder males.

1. Subjects and methods

1.1. Subjects

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 h light:dark cycle, which began at 0800 h. Female Long–Evans rats (Harlan) were housed overnight with proven breeder Long–Evans

males. Vaginal smears were conducted the following morning to check for the presence of sperm. The first day on which sperm was detected on the smear was designated gestational day (GD) 1. On GD 1, dams were individually housed in polypropylene cages with wood shavings and assigned to a treatment group. Three treatment groups were used in this study: ET (ethanol-treated), IC (intubated control), and NC (nontreated control).

1.2. Prenatal treatment

Ethanol administration to dams was performed from GD 1 through GD 22 in the latter half of the light cycle. ET dams were weighed daily and received daily intragastric intubations of ethanol (4.5 g/kg) in a volume of 20 ml/kg of distilled water from GD 1 to 22. Intubations consisted of insertion of a stainless steel gavage tube down the esophagus of the rat and injection of the ethanol dose directly into the stomach. The tube was dipped in corn oil to provide lubrication. ET dams also were given ad libitum access to water and rat chow.

The rat chow was weighed daily to monitor food intake. An isocaloric maltose–dextrin solution in a volume of 20 ml/kg was administered to IC dams every day during gestation (GD 1–22) through intragastric intubation. IC dams were pair-fed to an ET dam matched for gestational day and body weight on GD 1. The handling time for the intubation procedures was 2 to 5 min. The NC dams were weighed daily, but did not receive any other treatments. Three hours after intubations on GD 20, 10 μ l of blood were collected from ET and IC dams via a nick to the tail. The blood samples from the ET dams were processed for determination of peak blood ethanol concentrations (BECs). No blood was taken from NC dams. The day of birth (GD 23) was designated postnatal day (PD) 1. Neither the dams nor the pups received any treatment on this day.

1.3. Postnatal treatment

Litters were culled to 10 pups (5 male, 5 female) whenever possible. On PD 2 through PD 10, pups from all groups were removed from the nest one at a time and weighed, marked with nontoxic marker for identification, and intubated (ET and IC groups). All pup intubations were performed using PE 10 Intramedic tubing dipped in corn oil for lubrication and conducted in the latter half of the light cycle. ET pups received a 3.0 g/kg dose of ethanol in a volume of 27.8 ml/kg milk [24]. Two hours after the ethanol administration, ET pups were intubated a second time with the milk solution only (27.8 ml/kg) to compensate for any reduction in milk intake due to intoxication. The milk solution was formulated to simulate dam's milk [24]. The IC pups received the same procedure (two intubations) as the ET pups with PE 10 Intramedic tubing dipped in corn oil, but no solutions were administered. The postnatal procedure duration was approximately 2 min for each pup, and every effort was made to reduce the time of separation between the pup and the dam. The NC pups were weighed and handled only once daily in the latter half of the light cycle from PD 2 through 10. For both rounds of intubations, each pup was removed from the litter individually and treated accordingly. Two hours after intubations on PD 10, 10 μ l of blood was collected via tail clip from ET and IC pups. The blood samples from the ET pups were processed for determination of peak BECs. No blood was taken from NC pups. Pups were weaned on PD 21 and housed with a same-sex cage mate from the same litter (and thus, same treatment group) in a clear, polypropylene cage with wood shavings.

1.4. Blood Ethanol Concentrations (BECs)

Peak BEC is suggested to be a more important determining factor in the teratogenic effects of ethanol than the total time of exposure [25,26]. The doses of ethanol used in this study (4.5 g/kg for dams and 3.0 g/kg for pups) were determined by pilot studies conducted in this laboratory to give equivalent peak BECs in dams and pups and to give BECs in a range seen in alcoholic women [27]. The BEC in the dam has been shown to reflect the BEC in the

fetus [28]. The timing for sampling peak BECs in dams and pups was determined by a previous time-course study [27]. The blood samples from ET pups and dams were immediately placed in 190 ml of 0.53 N perchloric acid, neutralized with 200 ml of 0.30 M potassium carbonate, vortexed, and then centrifuged (Mikro 22R Hettich Zentrifugen) for 15 min. They were then frozen at -80°C until the assays were performed — a period of time ranging from between 1 week to 6 months. The variability introduced by the differences in freezing time was controlled by setting up and freezing a set of standards in parallel with each blood sample. BECs from ET dams and pups were determined using an enzymatic procedure [29].

1.5. Aggression tests

Aggression testing was conducted between 90 and 130 days of age between 0800 and 1000 h. Aggression testing utilized the procedures from the resident/intruder paradigm (as described in [23]) with the experimental animal as the resident. Each experimental animal was tested under the three housing conditions. A different age- and weight-matched intruder was used in each housing condition. The housing conditions were (1) with another male, (2) with a pregnant female, and (3) with a female and litter fathered by the experimental animal. Aggression was first assessed in experimental males housed with the same male since weaning. After the first test of aggression, the experimental male was housed with a non-experimental female for breeding. The female was checked for pregnancy by vaginal smears. The first day sperm was present was assigned as GD 1. During gestation (between GD 13 and 17), aggression was assessed a second time. Finally, after the birth of pups to the cohabitating female, a final session of intermale aggression was conducted when the pups were 7 days of age. The experimental animal was always the resident and the intruder was a novel non-experimental male of similar weight and age.

The testing was the same for each condition. The cage mate(s) of the experimental resident were removed and an unfamiliar intruder was placed in its home cage and their behavior was videotaped for 5 min or until the first attack bite. Duration of offensive aggressive behaviors of the resident and defensive aggressive behaviors of the intruder was recorded. Offensive behaviors included pursuit/chasing, sideways threat postures and movements, pinning/pushing, boxing, and attack bites. Defensive behaviors included escape reactions, defensive upright postures, crouching, and audible vocalizations. In addition, durations of other social behaviors, including anogenital contact, naso-nasal contact, and social grooming were measured.

1.6. Pain sensitivity

On the day after the last aggression test, experimental males were tested for pain sensitivity using the tail-flick test (as described in [30]), in order to determine if group differences in acute pain sensitivity could account for differences in aggressive behavior. Ten centimeters of the rat's tail was immersed in 55°C water and latency for rat to withdraw its tail was measured. Testing consisted of four consecutive trials separated by 10 min with a 10 s ceiling on the withdrawal response. The latency for each animal was an average of the latencies across the four trials.

1.7. Testosterone levels

Immediately after testing for aggression, the subjects were housed in the same pairs that existed prior to the beginning of the experiment. At least seven days after the conclusion of aggression testing, rats were killed by decapitation and trunk blood was collected and frozen at -80°C . After thawing, the blood sample was centrifuged and serum was extracted for the assay. For the testosterone radioimmunoassay, the DPC solid phase Coat-A-Count® procedure was used (Diagnostic Products Corporation, TKTT1, Los Angeles, CA), with

standards ranging from 20–1600 ng/dl testosterone. Briefly, triplicate 50 μ l aliquots of samples or standards were added to polypropylene antibody-coated tubes and incubated with 1 ml of 125 I-testosterone. After 3 h at 37 °C, the liquid was aspirated off, the tubes were allowed to drain several minutes, and tubes were counted in a gamma counter for 1 min. Amounts of testosterone in the samples were determined by comparison with the standard curve using log–logit transformation of the data.

2. Results

2.1. Body weights

There were 16, 19, and 17 dams in the ET, IC, and NC groups, respectively; one male from each litter was used in this study. There were no significant differences among groups with respect to litter size (overall mean number of pups and standard error of the mean (SEM)=9.25 $\pm$ 0.16) and sex ratio (overall mean and SEM of number of males to females=1.14 $\pm$ 0.09).

Dam body weights on GD 1 through GD 22 were analyzed using a repeated measures analysis of variance (ANOVA). Only a main effect of gestational day [$F(21,1029)$ =295.0; P <.001] was found indicating a normal increase in weight due to pregnancy for all groups. Body weights of the dams on GD 1, 5, 10, 15, 20 and 22 are shown in Table 1.

A repeated measures ANOVA on the body weights on PD 2 through 10 of those animals tested in the current study indicated significant main effects of group [$F(2,49)$ =24.9; P <.001] and day [$F(8,292)$ =1907; P <.001] and a significant interaction of group and day [$F(8,392)$ =1907; P <.001]. In general, ET animals weighed less than the IC and NC animals (Tukey's HSD test: P s<0.05) and all animals gained weight from PD 2 through 10. Further analyses of simple main effects followed by Tukey's HSD tests indicated that the ET animals weighed significantly less than the IC and NC animals, which did not differ from each other, on PD 2 through PD 10 [P s<.05]. A repeated measures ANOVA on the body weights on PD 21, 30, 60 and 90 of those animals tested in the current study revealed a significant main effect of postnatal day [$F(3,147)$ =3377; P <.001] (indicating weight gain) but no effect of or interaction with group. Body weight data of the offspring on PD 2, 10, 21, 30, 60 and 90 are depicted in Table 2.

2.2. Blood ethanol concentrations

Average BECs and standard error of the means (SEMs) of the dams and pups were 310 $\pm$ 21 and 265 $\pm$ 23 mg/dl, respectively. There was no significant difference between dam and pup BECs.

2.3. Aggression tests

The resident experimental males exposed to ethanol showed reduced offensive aggression when housed with a female and pups and did not show changes in non-aggressive social behavior compared to the control males (see Fig. 1). A repeated measures ANOVA on the offensive behaviors of the resident experimental males across housing conditions indicated an interaction between housing condition and group [$F(4,98)$ =4.9; P <.01]. Analyses of simple main effects on each condition indicated a significant effect of group only when the testing was done on experimental animals housed with a female and her pups [$F(2,49)$ =5.8; P <.01]. This effect was due to the ET males exhibiting less offensive aggression than either the IC or NC males (Tukey's HSD; P s<0.05). The IC and NC males did not differ from each other. A repeated measures ANOVA on non-aggressive social behavior of the resident experimental males across housing conditions revealed no significant effects (see Table 3).

There was not enough defensive aggression by the resident experimental males to yield a valid analysis; this data is shown in Table 3.

Biting, which was a behavior that was included in the category of offensive aggression, was examined separately. None of the 16 ET animals in any of the housing conditions engaged in biting. In contrast within the IC group of 19 animals, there were 3, 2, and 1 animal(s) which ended an aggression test session by biting when housed with a male, with a pregnant female, or with a female and pups, respectively. With the NC group of 17 animals, there were 2, 3 and 6 animals which ended an aggression test session by biting when housed with a male, with a pregnant female or with a female and pups, respectively. It is clear that the reduction in offensive aggression by the ET group could not result from a shorter period of observation that would result from a quick latency to a bite (since there were no bites by members of this group). The complete lack of biting in the ET group is consistent with our finding of lower levels of offensive aggression generally.

The pattern of findings in the intruder males reflected that seen in the resident males except that the reduction was observed in defensive aggression. A repeated measures ANOVA on the defensive aggressive behaviors of the intruder males across housing conditions indicated an interaction between housing condition and group [$F(4,98)=3.80$; $P<.05$]. Analyses of simple main effects on each condition indicated a significant effect of group only when the testing was done on intruders tested with an experimental animal housed with a female and her pups [$F(2,49)=4.76$; $P<.05$]. Intruders tested with ET males exhibited less defensive aggression than intruders tested with either the IC or NC males (Tukey's HSD; $P<.05$), which did not differ from each other (see Fig. 2). A repeated measures ANOVA on non-aggressive social behaviors of the intruder males across housing conditions revealed no significant effects (see Table 3). There was not enough offensive aggression by the intruder males to yield a valid analysis and none of the intruder animals exhibited any biting in any test session. These data are shown in Table 3.

2.4. Pain sensitivity

An ANOVA on the average tail flick latencies revealed no significant effect of group. Average tail flick latencies and SEMs for the ET, IC and NC groups were 2.74 ± 0.21 , 2.16 ± 0.23 , and 3.02 ± 0.35 , respectively.

2.5. Testosterone levels

A univariate ANOVA on the testosterone levels indicated a main effect of group [$F(2,49)=21.2$; $P<.05$]. The testosterone levels in the ET animals were significantly lower than those in the IC and NC animals (Tukey's HSD; $P<.05$) (see Fig. 3). The correlation of testosterone levels to offensive aggression is 0.25, which is a small but significant ($P<.05$) relationship.

3. Discussion

The results confirm the original hypotheses. This study found that ethanol exposure during a period equivalent to three trimesters of human development reduced offensive aggression in a resident/intruder paradigm and reduced testosterone levels in male rats compared to both controls groups. A non-experimental intruder tested with ethanol-exposed males showed a concomitant decrease in defensive behavior compared to both control groups. Non-aggressive social behaviors and pain sensitivity did not differ among groups.

The reduction of inter-male aggression by ethanol exposure compared to both control groups has to our knowledge only been found in mice [6]. The reduction of aggression may critically depend upon the exposure period; Yanai and Ginsburg [6] found a decrease in

aggression following postnatal exposure, whereas studies using prenatal exposure periods found an increase in aggression [4-7]. Administration of ethanol during a combined prenatal and postnatal period produces different effects on activity, brain weight, and spatial performance than administration during a shorter period [21,22,31]. In the current study duration of non-aggressive social interactions and tail flick latencies were not affected, suggesting that the effect of ethanol exposure was specific to aggression and not a general reduction in social interaction (such as that seen in [32]) or a change in acute pain sensitivity. Interestingly, there was no apparent reduction in aggression due to ethanol in conditions where aggression was relatively low (i.e., in conditions where the experimental male was housed with another male or housed with pregnant female). This may be due to a floor effect, particularly in the condition where the housing was with another male. Alternatively, it is possible that the experimental animal failed to respond with increased aggression when the context, such as the protection of a female with offspring from an intruder, demanded an aggressive response.

These findings support other studies that have found a decrease in testosterone in ethanol-treated adult rats [10] and adult mice [33]. Because testosterone levels were measured in adulthood after mating and three aggression tests, it is difficult to determine the mechanism behind the reduction of testosterone levels. One possibility is that the ethanol-exposed animals experience more defeat during the aggression tests or during their lifetime (since they are housed in pairs) and this experience results in a lower testosterone level [3,15,34,35]. However, the ethanol-exposed animals were housed with an animal also treated with ethanol, making it unlikely that there are long-term differences across groups in social defeat. Further, the aggression levels in the intruders tested with the ethanol-exposed animals were also lower than intruders tested with the control animals, suggesting an overall reduction in aggression and not an increase in social defeat by the intruders. Ethanol exposure during development reduces the testosterone surge during the early postnatal period in rats [36] and reduces the size of the testes [10]. The decrease in testosterone during development may result in males that are less masculinized, and there is strong evidence that a number of sexually dimorphic behaviors are indeed less masculinized in ethanol-exposed animals. For example, the change in testosterone levels may contribute to the female-like juvenile play [37] and active social interactions [32], the feminized patterns of social partner preference [38], and the decrease in the ano-genital distance [10] seen in ethanol-exposed males. Ethanol-exposed males are more likely to exhibit lordosis behavior [39] and are less likely to ejaculate [9]. The decrease in masculinization may also be responsible for the decrease in aggression behavior seen in the current study, since female rats typically show low levels of aggression [14,40]. Nevertheless, in order to begin to understand the mechanism of the ethanol-induced reduction in adult testosterone levels, a developmental time course describing the changes in testosterone over the lifespan is necessary.

Ward and colleagues [11,12,41] have repeatedly shown that the effects of prenatal ethanol exposure often synergize with prenatal stress to affect measures of male sexual behavior and reduce testosterone levels during development. In particular, they found that while prenatal stress suppresses prenatal and does not affect postnatal testosterone, ethanol exposure enhances prenatal and suppresses postnatal testosterone levels [11,12,41]. Prenatal exposure to ethanol and stress combined have the most dramatic feminizing effect on male sexual behavior [9]. The current ethanol administration procedures involve intubations during the prenatal and postnatal periods and two tail nicks (one during each of the prenatal and postnatal period) and intubations and tail nicks are clearly acute stressors. While we show no effects of intubation alone on any of the measures (as shown by the lack of differences between the IC and NC group), it is possible that the stressor effects are sub-threshold and that there is an interaction of the daily acute stressor of intubation with ethanol to produce some of the observed effects, particularly the reduced testosterone levels. This possibility

exists for all ethanol administration procedures since all of them produce some degree of stress on the animal, and it must remain a caveat in the interpretation of the effects found in animal models of FASD. Another caveat is that the weight reduction from PD 2 through 10 observed in the ET animals may contribute to the effects of ethanol in the present study. However, the observed weight reduction is due to ethanol exposure during the second half of gestation [22] and is not a result of undernutrition since there are no body weight differences across dam groups. Thus, dissociating the effect of ethanol on the development of the body from that on the brain is not possible at least in an in vivo model.

The lack of findings on pain sensitivity is somewhat surprising because previous studies have found that testosterone levels do relate to pain sensitivity and anxiety [42,43]. Interestingly, low levels of testosterone have been shown to enhance the fear response as measured by freezing and fear-induced analgesia [44], suggesting the possibility that the ET animals in the current study may show reduced aggression because of enhanced fear. However, it would then be expected that there be a reduction in social behavior generally in the ET animals, since social interaction is used as an index of anxiety, and this was not the case. These inconsistencies might also be related to the distinct testing paradigms used in each study, since prior stress can modulate pain sensitivity.

This study has shown that prenatal and postnatal ethanol exposure results in decreased aggression and decreased testosterone in adult male rats. The effects of combined prenatal and postnatal ethanol exposure are more likely to reflect the human condition of FASD than shorter periods of exposure. FASD individuals are reported to be more likely to have problems with the law [1], but the current findings, if generalizable to humans, would suggest that FASD individuals are not more aggressive and that their social difficulties stem from other deficits. Trouble with the law and social difficulties in general could result from poor judgment, failure to appreciate the consequences of actions, poor home environments, or a large discrepancy between I.Q. and level of adaptive behavior [1]. Nevertheless, the current findings do suggest that ethanol exposure during development does result in differences in complex social behaviors and the social domain generally. Future studies of those with FASD and animal models of FASD are needed to more fully delineate the nature of the social abnormalities induced by ethanol exposure during development.

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Fig. 1. Duration of offensive aggression in resident experimental males across housing conditions. The star indicates a significant difference between the ET group and the control groups in that condition. Error bars represent standard error of the mean (SEM).

Fig. 2.

Duration of defensive aggression in intruder males across housing conditions. The group and housing condition of the intruder male is determined by the status of the resident experimental animal. The star indicates a significant difference between the ET group and the control groups in that condition. Error bars represent SEM.

Fig. 3. Plasma testosterone levels in experimental animals. The star indicates a significant difference between the ET group and the control groups. Error bars represent SEM.

Table 1

Mean body weights of dams (g) and SEMs

Group	Gestational day					
	1	5	10	15	20	22
ET	263.0±1.1	271.1±1.3	285.1±1.3	307.1±1.1	358.0±3.3	364.9±1.2
IC	258.7±1.0	261.7±0.8	272.0±0.8	288.8±0.7	328.4±1.0	346.7±1.2
NC	255.8±0.8	257.6±0.9	267.9±0.7	288.1±0.7	329.7±1.0	350.1±1.3

Table 2

Mean body weights of the experimental animals (g) and SEMs

Group	PD 2	PD 10	PD 21	PD 30	PD 60	PD 90
ET	6.3±0.3	18.4±0.6	48.2±1.2	93.5±1.9	310.0±1.3	431.6±1.5
IC	7.1±0.2	20.2±0.3	47.8±0.6	107.8±5.4	324.4±1.0	452.5±1.4
NC	7.4±0.2	20.8±0.5	46.3±0.9	94.6±0.6	320.0±1.6	435.3±1.7

Social behavior other than aggression, defensive aggression by the resident animals, and offensive aggression by the intruder animals

Table 3

	Duration of social behaviors other than aggression (mean±SEM (s))			Resident animals showing defensive aggression or Intruder animals showing offensive aggression (%)		
	Housing with			Housing with		
	Male	Pregnant female	Female and pups	Male	Pregnant female	Female and pups
<i>Resident groups</i>						
ET	12.5±2.5	19.4±3.6	17.8±2.4	25.0	18.7	0.0
IC	14.7±4.6	13.6±1.6	15.2±2.2	15.8	5.3	5.3
NC	11.9±2.1	16.9±2.8	21.4±3.2	23.5	23.5	23.5
<i>Intruder groups</i>						
ET	9.7±1.9	6.9±1.4	4.1±1.0	25.0	25.0	6.3
IC	6.9±1.5	5.6±1.3	11.0±4.8	21.0	26.3	5.3
NC	9.6±2.6	6.6±1.6	5.1±1.1	23.5	35.3	29.4