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Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment

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Abstract

Epigenetic mechanisms such as DNA methylation are part of an emerging story on how early-life experiences can alter behavioral trajectories and lead to the development of disease and psychological disorders. Previous work from our laboratory has demonstrated alterations in methylation of DNA associated with the *brain-derived neurotrophic factor* (*bdnf*) gene within the amygdala and hippocampus of infant and adult rats that were repeatedly exposed to caregiver maltreatment outside the home cage during their first week of life. In the current study we examine changes in global levels of DNA methylation (5-mC) and hydroxymethylation (5-hmC), as well as gene-specific changes in methylation patterns of the candidate gene *bdnf* (at exons I and IV) within the adolescent amygdala and hippocampus resulting from exposure to maltreatment. While adolescent females exposed to maltreatment showed no significant alterations in global 5-mC or 5-hmC levels, examination of *bdnf* DNA methylation revealed that maltreated-females had greater methylation of exon IV DNA in the amygdala and ventral hippocampus. While adolescent males exposed to maltreatment showed no significant alterations in *bdnf* DNA methylation, maltreated-males had significantly higher 5-mC levels in the dorsal hippocampus and lower 5-hmC levels in the amygdala. These findings demonstrate that the effects of the early caregiving environment are detectable in the adolescent brain at the level of the epigenome, with brain-region specific and sexually-dimorphic epigenetic consequences that could have relevance to adolescent mental health and behavior.

Keywords

DNA methylation; DNA hydroxymethylation; early-life stress; *bdnf* gene; adolescence

1. Intro

It is widely recognized that the early postnatal environment, specifically within the context of the caregiving relationship, moderates the development of behavior and disease with

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early, adverse experiences conferring vulnerability to aberrant brain development, dysregulation of immune function, anxiety and mood disorders, deficits in learning and memory, and a myriad of other consequences that persist throughout the life of the organism [1–10]. While the consequences of disruption to the caregiving relationship are better known, the mechanisms by which these consequences occur are less understood. One proposed mechanism is DNA methylation, an epigenetic modification that occurs via the addition of methyl groups to cytosines (5-mC), typically within cytosine-guanine dinucleotides. This modification is generally associated with a decrease in gene transcription as it can both impede the binding of transcription factors and recruit repressor proteins that can condense chromatin (as reviewed in [11–14]). This is a dynamic process that varies by age, sex, and brain region, and one that is responsive to both pre- and postnatal adversity [11, 15–19].

Recently, another modified form of cytosine has been gaining attention as an epigenetic modification that may play a significant role in development and behavior [20]. A derivative of 5-mC, 5-hydroxymethylcytosine (5-hmC) occurs through an oxidative process that is catalyzed by the ten-eleven translocation (TET) family of proteins [21]. While it has been proposed that this oxidized form of methylcytosine is simply an intermediary in the process of demethylation, more recent evidence suggests it encompasses a larger role and may instead be a stable epigenetic modification [22–24]. For example, it is known to increase in concentration in neuronal cells as an organism ages [20], is responsive to changes in neural activity [23] including learning tasks such as fear extinction training [25], and is enriched in the brain and in genes related to synaptic function, implying a pivotal role in psychiatric disorders [26]. It has also been shown to inhibit methyl-CpG binding protein 2, a methyl binding protein important in the process of methylation, and is therefore posited to play a meaningful role in gene expression [27]. It is likely that 5-hmC functions in both capacities (as an intermediate and stable modification, both able to influence gene expression), and regardless of its exact role is a modification that requires more attention in developmental research.

Often, studies addressing the link between maternal behavior and/or adverse early environments and epigenetic modifications have focused on gene-specific changes in methylation. In this study, however, we chose to begin our investigation at a global level. While a handful of studies have examined global 5-mC levels following various forms of early-life stress [28–31], the results have been inconsistent and the most commonly examined time point has been in adulthood. For these reasons, we deemed it necessary to determine global alterations of both 5-mC and 5-hmC in the adolescent brain occurring in response to our maltreatment regimen.

As stated, a more heavily used approach has been examination of gene-specific DNA methylation [15–17, 32–40]. This includes methylation of the *brain-derived neurotrophic factor (bdnf)* gene—a critical player in development and synaptic plasticity [41, 42] that is known to exhibit environmentally-driven epigenetic changes, particularly in response to stress or quality of caregiving [19, 37, 43, 44]. While previous work from our laboratory has uncovered alterations in methylation levels of DNA associated with this gene in both infants and adults following exposure to caregiver maltreatment [11, 18, 37], one age point that

remains to be investigated is adolescence, particularly in the amygdala and hippocampus. Our previous data also support the idea that exposure to maltreatment produces alterations in CNS DNA methylation that either persist or change with development (i.e. disappear or emerge with maturation). These changes are intriguing from a mechanistic point of view as the brain regions investigated are known players in the development and regulation of fear- and anxiety-related behaviors [45, 46], and their functions are known to be affected by stress [47]. These changes are also relevant in terms of psychiatric disorders considering that *bdnf* disturbances have been implicated in the pathology of anxiety-related disorders [48], depression [49], and PTSD [44, 50]. However, before we can confidently move forward in linking epigenetic alterations with behavioral trajectories, we must first understand precisely when and where these alterations are occurring—information that we currently lack for the adolescent brain in our model. Beyond extending our understanding of maltreatment-induced epigenetic alterations, investigation of the biological effects of early-life stress on the adolescent brain and the role those effects play in adolescent mental health is crucial, as it is estimated that one in five adolescents are affected by some type of psychiatric disorder that they will carry into adulthood [51].

Therefore, the current study examines global alterations in 5-mC and 5-hmC as well as gene-specific DNA methylation alterations in the adolescent rat amygdala and hippocampus in response to brief and repeated exposure to an adverse caregiving environment during infancy. We also compared patterns in both males and females, as there is increasing evidence of sexually-dimorphic epigenetic changes in response to early environmental factors [11, 15, 18, 52, 53].

2. Methods

2.1 Subjects and Caregiving Manipulations

Male and female outbred Long-Evans rats were housed in polypropylene cages in a temperature-controlled room on a 12-hour light/dark cycle with lights on at 6:00 am. All rats were given plenty of bedding and *ad libitum* access to food and water. Rats were bred and the day of parturition was designated Postnatal Day (PN) 0. All dams had given birth at least once prior to beginning the experiment in order to ensure that no first-time mothers were used. On PN1, litters were culled to 5–6 males and 5–6 females and split into three groups using a within-litter design. Beginning on PN1 and ending on PN7, groups (up to 4 pups—ideally 2 males and 2 females) were exposed to the maltreatment condition, the cross-foster care condition, or the normal-care condition. Pups in the maltreatment condition were exposed to a dam in a novel environment wherein the dam was given little nesting material and little time (five minutes) to habituate to the environment. Pups in the cross-foster care condition were exposed to a dam in a novel environment wherein the dam was given plenty of nesting material and ample time (one hour) to habituate to the environment. Pups in the normal-care condition were only exposed to the home cage and caregiving from their biological mother.

All sessions were live-scored by a trained observer and then scored again from video playback by a second trained observer. Both nurturing maternal behaviors (nursing, licking and grooming) and aversive maternal behaviors (stepping on, dropping, dragging, roughly

handling, or actively avoiding the pups) were tallied in five minute time bins (marking the occurrence or not), averaged across the 7 exposure days, and then an average of the two observers' scores was taken for statistical analysis (Table 1). Pup vocalizations (both audible and ultrasonic (40kHz)) were also recorded during each session and two trained individuals subsequently marked the occurrence (or not) of a vocalization during each minute time bin. The vocalizations were averaged across the 7 exposure days, and then an average of the two observers' scores was taken for statistical analysis (Table 1). These experimental procedures are similar to those used in our previous reports [11, 18, 37], were performed during the light cycle, and were approved by The University of Delaware Animal Care and Use Committee.

2.2 Global DNA Methylation and Global Hydroxymethylation Assays

Animals were sacrificed at baseline conditions (i.e. with minimal disturbance when taken from the home cage) at PN30. The amygdala (homogenate of basolateral, lateral, and central nuclei), dorsal hippocampus, and ventral hippocampus were dissected on dry ice. DNA was extracted according to the manufacturer's instructions (Qiagen AllPrep DNA/RNA kit) and stored at -80°C until further processing.

MethylFlash™ Methylated DNA Quantification Kits were used to quantify genome-wide methylation (5-mC) and hydroxymethylation (5-hmC) levels according to the manufacturer's instructions (Epigentek, Brooklyn, NY). Briefly, these assays employ capture and detection antibodies to detect either methylated or hydroxymethylated genomic DNA, which is then colorimetrically quantified (by measuring absorbance) against a standard curve consisting of positive (methylated polynucleotide containing 50% 5-mC or 20% 5-hmC cytosine) and negative (unmethylated polynucleotide containing 50% cytosine [5-mC kit] or 20% cytosine [5-hmC kit]) control DNA, which were provided with the kit. Absorbance was measured using the Infinite® F50 microplate reader (Tecan, Männedorf, Switzerland) with the amount of 5-mC or 5-hmC DNA being proportional to the intensity of the optical density.

The absolute amount of methylated DNA was calculated as follows: $5\text{-mC (ng)} = [\text{Sample OD} - \text{Negative control OD}] / [\text{Slope of the Standard Curve} \times 2]$, with OD being optical density and two being a factor to normalize the positive control to 100%. Using the resulting value, the amount of 5-mC content was calculated as follows: $5\text{-mC \%} = [5\text{-mC amount (ng)} / \text{input sample DNA (ng)}] \times 100\%$. The absolute amount of hydroxymethylated DNA was calculated as follows: $5\text{-hmC (ng)} = [\text{Sample OD} - \text{Negative control OD}] / [\text{Slope of the Standard Curve} \times 5]$, with OD being optical density and five being a factor to normalize the positive control to 100%. Using the resulting value, the amount of 5-hmC content was calculated as follows: $5\text{-hmC \%} = [5\text{-hmC amount (ng)} / \text{input sample DNA (ng)}] \times 100\%$.

Standard curves were prepared for both assays by diluting the positive standard to the following concentrations (ng/ul): 0.5, 1.0, 2.0, 5.0, and 10.0. Optical density readings were proportional to the amount of methylated or hydroxymethylated DNA in a sample, with the negative control reading at approximately .05, the first point in the positive control (0.5 ng/ul) reading between 0.1 and 0.3, and the remaining positive control readings

approximately doubling with each increase in concentration. Data were only used from assays where an r^2 value of .9 or higher was obtained for the standard curve.

2.3 Locus-specific DNA methylation

The same DNA used for global measures of 5-mC and 5-hmC was used for gene-specific assays. *Bdnf* methylation was assessed via methylation-specific real-time PCR or MSP (Bio-Rad CFX96 system). Primers designed to distinguish between methylated and unmethylated DNA [11, 18, 37, 54] were used to amplify bisulfite-modified DNA (Qiagen Inc.) associated with *bdnf* exons I and IV. The comparative C_t method was used to quantify the relative fold change of maltreated and cross-foster care animals versus normal care controls [55] and the methylation index was obtained by dividing the fold change value for the methylated primer set by the fold change value for the unmethylated primer set [11, 18, 56]. PCR reactions were performed in triplicates and product specificity was confirmed with melt curve analysis and gel electrophoresis. Additionally, verification of MSP results in the female ventral hippocampus was performed using direct bisulfite sequencing (BSP) as previously described [18, 37, 44, 57], allowing an estimate of methylation levels at each CG cite within the targeted *bdnf* IV region.

2.4 Statistical Analyses

Global and gene-specific data were analyzed using two-way ANOVAs (factors: infant condition and sex) and appropriate post-hoc tests. MSP data were further analyzed using one-sample *t*-tests for comparison with normal care controls (a mean value of 1 would indicate no change in levels in comparison to this control group). An independent sample *t*-test was used to compare methylation of collapsed CG sites (BSP data) between maltreated and normal care females. Outliers that fell more than two standard deviations above or below the mean were excluded (amygdala 5-mC: 1 maltreatment male; ventral hippocampus 5-hmC: 1 cross-foster male; dorsal hippocampus 5-hmC: 1 normal-care female; amygdala 5-hmC: 1 maltreatment male; ventral hippocampus MSP: 1 cross-foster female [exon I], 1 maltreatment male [exon IV]; amygdala MSP: 1 maltreatment female [exon I]). For all analyses, differences were considered to be statistically significant for $p < 0.05$.

3. Results

3.1 Global 5-mC and 5-hmC

As previously reported for this cohort of rats ([11] this study only examined the medial prefrontal cortex) and summarized in Table 1, pups assigned to our maltreatment condition were subjected to a greater proportion of aversive caregiving behaviors (i.e. being stepped on, dropped, dragged, actively avoided, and roughly handled) than pups assigned to either the cross-foster or normal care conditions (which experienced more licking and grooming, hovering and nursing behaviors). Further, they emitted significantly more audible and ultrasonic vocalizations than pups in either the cross-foster or normal care conditions (also summarized in Table 1).

To assess global methylation alterations resulting from exposure to an adverse caregiving environment, we investigated 5-mC and 5-hmC levels within the adolescent amygdala and

hippocampus. For the amygdala, two-way ANOVAs for 5-mC (Figure 1A) and 5-hmC (Figure 1B) levels revealed no main effects of sex or infant condition nor an interaction between these factors when all groups were present. Since lower 5-hmC levels were apparent in maltreated males and there was no difference in 5-hmC levels between the normal and cross-foster care groups (infant condition $p = 0.776$, sex $p = 0.126$, interaction $p = 0.966$), these groups were collapsed to enhance statistical power in the two-way ANOVA. A significant main effect of infant condition [$F(1,54) = 4.336$, $p = 0.042$] as well an interaction effect [$F(1,54) = 4.273$, $p = 0.044$] were found, with maltreated males having lower 5-hmC levels than controls ($p = 0.015$).

In the dorsal hippocampus for 5-mC levels (Figure 2A), a significant main effect of infant condition was found [$F(2,54) = 3.537$, $p = 0.036$]. Global 5-mC levels in this brain region were found to be approaching a main effect of sex [$F(1,54) = 4.010$, $p = 0.05$]. Further, a significant interaction between infant condition and sex was detected [$F(2,54) = 3.599$, $p = 0.034$], with maltreated males exhibiting higher levels of 5-mC than all other groups (all p -values < 0.05). 5-hmC levels in the dorsal hippocampus did not differ across infant conditions or sexes (Figure 2B). Using a two-way ANOVA to assess 5-mC levels in the ventral hippocampus (Figure 3A), we found a main effect of sex [$F(1,52) = 4.218$, $p = 0.045$] and a significant interaction between infant condition and sex [$F(2,52) = 3.311$, $p = 0.044$]. Post-hoc Bonferroni analyses revealed that maltreated males had significantly higher levels of 5-mC than maltreated females ($p = 0.009$), and maltreated females were found to have marginally lower levels of 5-mC than normal ($p = 0.076$) and cross-foster care females ($p = 0.096$). Finally, a two-way ANOVA for 5-hmC levels (Figure 3B) in the ventral hippocampus revealed no effects of infant condition, sex, or an interaction between these factors.

3.2 Locus-specific DNA methylation

We next investigated DNA methylation alterations of a gene in which our lab has previously found methylation differences following maltreatment. We examined DNA methylation associated with exons I and IV of *bdnf*, both of which are known to exhibit environmentally-driven epigenetic changes [37, 43, 58]. In the amygdala (Figure 4), significantly higher levels of methylation at *bdnf* exon IV were found in maltreated females in comparison to normal care controls ($t_8 = 2.413$, $p = 0.042$). Two-way ANOVAs for methylation of either *bdnf* exon I or IV revealed no significant main effects of sex or infant condition and no infant condition-sex interactions when comparing maltreatment and cross-foster care groups. No effects of infant condition, sex, or an interaction of these factors were detected for exons I or IV in the dorsal hippocampus, nor were any comparisons of maltreated animals to normal care controls significant (Figure 5).

The final region we examined with gene-specific assays was the ventral hippocampus. As illustrated in Figure 6A, maltreated females had higher levels of *bdnf* IV methylation in comparison to normal care controls ($t_8 = 2.795$, $p = 0.023$). Further, a two-way ANOVA revealed a main effect of sex for exon IV methylation in the ventral hippocampus [$F(1,29) = 5.488$, $p = 0.026$], with Bonferroni post-tests indicating significantly higher levels of methylation in maltreated females in comparison to maltreated males ($p < .05$). Finally, to

cross-verify our results, we used direct-bisulfite sequencing to assess *bdnf* methylation alterations at individual cytosine sites of exon IV DNA in the ventral hippocampus (Figure 6B; no main effect of CG site). Confirming MSP data, maltreated females had higher methylation across the 12 CG sites detected and amplified by our primers (Figure 6C; $t_{238} = 2.349$, $p = 0.0196$).

4. Discussion

The aims of the current study were to characterize patterns of global and gene-specific DNA methylation in the amygdala and hippocampus of adolescent rats that had been exposed to caregiver maltreatment during infancy. Our data indicate that DNA methylation patterns, both global and locus-specific, are altered in these adolescent brain regions as a result of these exposures. Further, we determined that these epigenetic alterations are brain-region specific and sexually-dimorphic. Specifically, maltreated males exhibited changes on a global scale with increased 5-mC levels in the dorsal (when compared to all other groups) and ventral (when compared to maltreated females) hippocampus, and decreased 5-hmC levels in the amygdala. On the other hand, maltreated females were affected on a gene-specific level, with increases in *bdnf* exon IV methylation in both the ventral hippocampus and amygdala.

While no studies to our knowledge have assessed the relationship between adolescent behavior and global 5-mC levels, literature suggests that levels of 5-mC are associated with abnormal behavioral trajectories such as increased drug-seeking behavior [29] and maladaptive stress responses [30] in adulthood. This confers validity to the connection between global methylation levels (often as a result of a disruption to the caregiving environment) and behavioral outcomes, a connection which has yet to be fully investigated across the lifespan. In the current study, we also found global alterations of 5-hmC levels. Again, literature indicates a connection between 5-hmC levels and behavioral outcomes, particularly in the context of learning and memory as evidenced by changes in 5-hmC distribution following fear extinction [25], which suggests that it would be worthwhile in future behavioral studies to investigate learning and memory in our rats.

Despite the advantage of the global assays to readily distinguish 5-mC and 5-hmC levels, drawbacks to these global measures exist. Not only are there a large number of genes involved in development, but each is likely to exhibit unique changes in methylation patterns in response to early stressors, and depending on the direction of those changes, effects could be washed out in a global assessment. For example, while maternal behavior is known to affect gene-specific methylation (e.g. *bdnf* [11, 18, 37], *Gad1* [39], *GR* [17], *Avp* [35], *ERα* [38]), a study examining global methylation in the hippocampus of adult rats found no significant changes in global patterns of 5-mC that reflected effects specific to the type of maternal care received in infancy [28]. The authors suggest that this evidence speaks in favor of maternal-care-induced methylation changes being highly gene-specific, a notion that our female gene-specific data (both past and current) support. Another important limitation of conducting these global assays is that they are unable to specify where in the genome methylation alterations have occurred, which makes it difficult to relate these data

to gene regulation as the majority of methylated CGs are not found within gene promoters [59, 60].

Due to these limitations, and because of prior data from our lab (to be discussed), we chose to also conduct a gene-specific investigation of methylation alterations in these brain regions by focusing on the *bdnf* gene, specifically at exons I and IV. We acknowledge that the MSP and BSP techniques we used here do not allow us to differentiate cytosine methylation versus cytosine hydroxymethylation for *bdnf* loci; however, we interpret the data as changes in DNA methylation, which is consistent with current interpretations in the field and previous reports. No significant differences in *bdnf* DNA methylation were found between groups or sexes at exon I in any of the adolescent brain regions investigated. At exon IV, the current data show us that methylation levels were significantly higher in the adolescent amygdala and ventral hippocampus of maltreated females, with no changes in the dorsal hippocampus. Regarding our past work with *bdnf* in these brain regions, we have previously found (when compared to normal-care controls) less methylation of *bdnf* exon I in the dorsal hippocampus and amygdala of maltreated males in adulthood while in the ventral hippocampus greater methylation levels were found at this exon in maltreated females in adulthood; none of these effects were found to be present in the infant brain [18]. Our previous work has also investigated changes at exon IV and found that, similar to exon I, there is less methylation in the adult dorsal hippocampus and amygdala of maltreated males, while in the ventral hippocampus there is greater methylation of exon IV in this group (maltreated males) and decreased methylation of exon IV in the adult amygdala of maltreated females (again, when compared to normal-care controls) [18]. These effects were also not found to be present in the infant brain [18]. However, the observation here of higher methylation levels of exon IV in maltreated females within the ventral hippocampus is an effect also seen in the infant ventral hippocampus [18]. Our work with the medial prefrontal cortex [11], which also investigated these three age points, revealed similar short-lived and later-emerging methylation alterations in the maltreatment group. Together, these data indicate that some alterations in methylation associated with early-life stress can become latent and present themselves with maturation.

This study extends the literature on the epigenetic consequences of early-life stress for the adolescent brain in both a global and gene-specific manner, and adds to a new, but growing body of literature about 5-hmC. To better understand the relationship between maltreatment-induced epigenetic modifications, related gene expression levels, and behavioral outcomes, future studies are necessary to investigate 5-mC/5-hmC changes at these *bdnf* loci, mRNA levels (both under baseline and activity-dependent conditions), and behavioral performance. Future studies will also need to investigate 5-hmC in infancy and adulthood in order to draw lifespan comparisons similar to those we have drawn thus far with methylation (5-mC). As epigenetic alterations have been implicated as a mechanism by which various aspects of the infant-caregiver relationship can affect later biological and behavioral outcomes [61–65], our data are suggestive that differential methylation of genes, including *bdnf*, could contribute to aberrant behavioral trajectories and/or development of psychiatric disorders, as well as the sex differences inherent within them. Given that we see changes unique to the

adolescent time point, it is possible that these patterns play a role in the prevalence of adolescent mental health and behavioral disorders.

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Highlights

- Maltreated-males had higher 5-methylcytosine levels in the hippocampus;
- Maltreated-males had lower 5-hydroxymethylcytosine levels in the amygdala;
- Maltreated-females had greater *bdnf* DNA methylation in the amygdala and hippocampus.

Figure 1. Global DNA methylation (A) and global hydroxymethylation (B) in the adolescent amygdala. $n=8-11$ /group; & $p < 0.05$ vs. collapsed control groups; error bars represent SEM.

Figure 2.

Global DNA methylation (A) and global hydroxymethylation (B) in the adolescent dorsal hippocampus. $n=9-11/\text{group}$; $*p < 0.05$ vs. all groups; $\#p < 0.05$ vs. maltreated-females; error bars represent SEM.

Figure 3. Global DNA methylation (A) and global hydroxymethylation (B) in the adolescent ventral hippocampus. $n=9-10/\text{group}$; $\#p < 0.05$ vs. maltreated-females; error bars represent SEM.

Figure 4. Differences in *bdnf* methylation at exons I and IV in the adolescent amygdala. n=9–10/group; * $p < 0.05$ vs. normal care controls; error bars represent SEM.

Figure 5. Differences in *bdnf* methylation at exons I and IV in the adolescent dorsal hippocampus. n=8–9/group; * $p < 0.05$ vs. normal care controls; error bars represent SEM.

Figure 6.

Differences in *bdnf* methylation at exons I and IV in the adolescent ventral hippocampus (A). Differences in methylation of each CG site (B) and average CG methylation (C) of *bdnf* exon IV DNA in normal care and maltreated-females. n=8–9/group; * $p < 0.05$ vs. normal care controls; # $p < 0.05$ vs. maltreated-males; error bars represent SEM.

Table 1

Nurturing and aversive behaviors and audible and ultrasonic vocalizations between infant conditions.

Pup-Directed Maternal Behavior	Normal-Care	Cross-Foster	Maltreatment	F	p
<i>Lick/Groom</i>	32.1% **	34.9% ***	15.3%	14.94	<.001
<i>Hover/Nurse</i>	53.7% ***	50.7% ***	26.2%	19.92	<.001
<i>Step On</i>	5.2% ***	6.6% ***	17.9%	21.39	<.001
<i>Drop</i>	1.3% ***	1.2% ***	6.5%	14.99	<.001
<i>Drag</i>	3.7%	3.2%	5.0%	2.29	.112
<i>Actively Avoid</i>	.1% ***	.6% ***	15.5%	36.61	<.001
<i>Roughly Handle</i>	3.9% ***	2.8% ***	13.6%	27.04	<.001
<i>Audible Vocs</i>	34.33% *	28.07% **	52.63%	7.29	<.01
<i>Ultrasonic Vocs</i>	41.59% ***	48.29% ***	85.91%	110.5	<.001

*
p < .05,

**
p < .01,

p < .001 vs. maltreatment group. No significant differences were found between normal-care and cross-foster care. Data from [11].