Long-Term Depression In Vivo: Effects of Sex, Stress, Diet, and Prenatal Ethanol Exposure

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ABSTRACT: Long-term depression (LTD) of synaptic efficacy has proven a difficult phenomenon to examine in vivo, despite the ease with which it is induced in a variety of in vitro preparations. Prior exposure to an acute stressful episode does however seem to enhance the capacity of the hippocampus to exhibit LTD in vivo in male animals. In the present experiments, we examined the capacity for low-frequency stimuli (low-frequency stimulation (LFS)) to induce LTD in juvenile male and female animals following an acute stress episode. Interestingly, prior exposure to stress was only required for the induction of LTD in male animals, while both control and stressed female animals exhibited equivalent LTD. In animals that were exposed to ethanol in utero, a similar requirement for prior exposure to stress to elicit LTD was found for male, but not female animals. This prenatal ethanol exposure did not in itself after the capacity for LTD induction in either sex; however, in utero food restriction did enhance LTD induction in both male and female animals, irrespective of whether they were exposed to stress just prior to being administered LFS. These results indicate that in utero dietary restriction more drastically affects CA1 LTD than in utero ethanol exposure. In addition, female animals seem to exhibit LTD in vivo in the absence of stress much more easily than their male counterparts.

KEY WORDS: prenatal ethanol; long-term depression; in vivo

INTRODUCTION

Long-term depression (LTD) of synaptic efficacy is reliably induced with low-frequency stimuli (LFS) in the hippocampus in vitro (Dudek and Bear, 1993; Christie et al., 1996, 1997; Doyere et al., 1996) and has also been observed in vivo (Heynen et al., 1996), though with less reliability than it is observed in vitro (Staubli and Scalfi, 1997; Fox et al., 2006). Interestingly, prior exposure to acute stress enhances the capacity of LFS to induce LTD (Xu et al., 1998; Xiong et al., 2003, 2004) while simultaneously reducing the capacity for long-term potentiation (LTP) in the hippocampus (Xu et al., 1997, 1998; Xiong et al., 2004). Thus, it may be that stress shifts the induction threshold for synaptic plasticity away from LTP to favor LTD. If this is the case, it would suggest that an exaggerated stress response might further enhance the capacity for LTD.

In humans and animals, fetal alcohol syndrome and fetal alcohol spectrum disorder (FAS and FASD, respectively) can encompass a variety of physiological abnormalities and deficits, including mental retardation (Abel and Sokol, 1986; Marcus, 1987; Spohr et al., 1993; Wattendorf and Muenke, 2005). Human and animal studies also indicate that in utero ethanol exposure negatively impacts hippocampal structure and function (Berman and Hannigan, 2000), resulting in impaired hippocampal-dependent learning as well as a reduced capacity for LTP (Swartzwelder et al., 1988; Sutherland et al., 1997; Richardson et al., 2002; Christie et al., 2005). Animals with prenatal ethanol exposure (PNEE), an animal model of FAS/FASD, can exhibit a heightened stress response (Taylor et al., 1982; Weinberg and Gallo, 1982; Weinberg, 1988; Weinberg and Petersen, 1991; Kim et al., 1999; Streissguth and O’Malley, 2000; Gabriel et al., 2001; Bertrand et al., 2005), suggesting that stress-induced changes to synaptic plasticity might be more apparent in these animals. The current study will first investigate the capacity for both juvenile male and female animals to exhibit LTD in the hippocampal CA1 region in vivo, following acute exposure to stress. In addition, the effects of PNEE and food restriction (a control group required for PNEE studies) on LTD in male and female animals will also be investigated.

METHODS

Animals and Mating

All animals used in this study were generated in the animal care colony of the Department of Psychology at the University of British Columbia. Twenty-eight virgin female Sprague-Dawley rats were paired with age-matched males (250–275 g; University of British Columbia Animal Care Services) and individually housed in polycarbonate cages (46 × 24 × 20 cm3) with Carefresh contact bedding (Absorption Corp., Bellingham, WA) for a 1-week adaptation period. The breeding colony was kept at a constant temperature of 21 °C and lights turned on from 7:00–19:00 h. After the adaptation period, males and females were paired in suspended wire mesh cages (63 × 24 × 18 cm3) that were...
Diet Administration

The in utero group assignments and feeding schedules were performed using procedures that are well established to produce control, dietary-restricted, and ethanol-exposed animal groups (Keiver et al., 1996, 1997; Keiver and Weinberg, 2003, 2004). On GD1, pregnant females were individually housed in polycarbonate cages and assigned to one of three feeding groups: (i) The ethanol group, in which females were given ad libitum access to a liquid diet containing ethanol (35.5% ethanol-derived calories; 6.61% v/v), (ii) the paired group consisting of a similar liquid diet as the ethanol group, but with an iso-caloric substitution of maltose-dextrin for ethanol; paired dams were offered a quantity of food that matched the amount of food consumed in g/kg by an ethanol dam on the corresponding day of gestation; and (iii) ad libitum animals that were given ad libitum access to standard rat chow. All groups had ad libitum access to water throughout gestation. The ethanol and paired diets were administered from GD1 to GD22. The ethanol dams were slowly introduced to the ethanol during the first 3 days of gestation by combining 1/3 ethanol diet with 2/3 paired diet on GD1, 2/3 ethanol diet with 1/3 paired diet on GD2, and 3/3 ethanol diet from GD3 to GD21. On GD22, liquid diets were replaced with ad libitum access to standard rat chow. All groups had ad libitum access to water throughout gestation. The ethanol and paired diets were administered from GD1 to GD22. The ethanol dams were slowly introduced to the ethanol during the first 3 days of gestation by combining 1/3 ethanol diet with 2/3 paired diet on GD1, 2/3 ethanol diet with 1/3 paired diet on GD2, and 3/3 ethanol diet from GD3 to GD21. On GD22, liquid diets were replaced with ad libitum access to standard rat chow to reduce any further deleterious effects of ethanol exposure on offspring (Weinberg, 1989). The ethanol and paired diets contained all of the necessary nutrients to provide adequate nutrition to females in both conditions, despite the decrease in the total amount of diet consumed when compared with the ad libitum animals (Weinberg, 1985). The liquid diets were obtained from Dyets (Bethlehem, PA) and are sold as Weinberg/Keiver high protein liquid diet-control (no. 710109) for the paired diet and Weinberg/Keiver high protein liquid diet-experimental (no. 710324) for the ethanol diet. To determine consumption, the diet bottles were replenished with freshly prepared diets and administered in the late afternoon prior to lights out, which helps to prevent a shift in the CORT circadian rhythm that can be observed in animals fed a restricted diet (Weinberg and Gallo, 1982).

During pregnancy, females were weighed on GD 1, 7, 14, and 21 while their cages were being cleaned, so that the animals were minimally disturbed. The day on which females gave birth was indicated as postnatal day 1 (PND1). On PND2, litters were culled to 10 pups (5 males and 5 females) and dams and pups were weighed on PND8, PND 15, and PND22, again during cage cleaning. During this postnatal period, all animals were given the same ad libitum rat chow diet so that maternal diet was not different between groups at this point. The pups generated by these procedures were then weaned and group-housed according to sex on PND22, and also allowed ad libitum access to rat chow. It is important to note that these animals were never exposed to ethanol or dietary restriction after they were born, and it is these animals, and not the dams, that were used for the subsequent electrophysiological studies between PND30 and PND35.

Blood Ethanol Concentration Measurements

To determine the maternal blood ethanol concentrations (BECs) during pregnancy, a tail blood sample was acquired on GD15 2 h after the presentation of the ethanol diet. These samples were obtained from a separate group of ethanol dams from those whose offspring were included in the study so that the stress of the procedure would not impact the experimental animals. Blood was collected and allowed to sit at 4°C overnight and centrifuged at 3,500 rpm for 10–15 min and serum was stored at −20°C until assay. BECs were measured using an alcohol reagent kit (no. A7504–150) and alcohol standard (no. A7504-STD) from Pointe Scientific (Lincoln Park, MI); assay was performed according to manufacturer’s instructions.

Stress Protocols

On the day that electrophysiological recordings were being made, the offspring were randomly assigned to naive or stress conditions, with the experimenter blind to the animal group designation. The stress condition involved placing a single rat on an elevated platform (10 × 10 cm² and 1.6 m high) in the middle of a brightly lit room for 30 min (Xu et al., 1998; Xiong et al., 2003, 2004). After this period, the animal was immediately anesthetized and a tail sample of blood was obtained to determine corticosterone levels. To analyze corticosterone levels, blood was collected in Fischer microcentrifuge tubes and centrifuged at 6,000 rpm for 10–15 min. Serum was collected and stored at −20°C until assay. Groups were identified in the following manner: male (n = 8) and female (n = 13) ad libitum naive animals; male (n = 10) and female (n = 9) ad libitum stress animals; male (n = 11) and female (n = 13) paired naive animals; male (n = 9) and female (n = 9) paired stress; male (n = 10) and female (n = 10) ethanol-naive animals; and male (n = 9) and female (n = 7) ethanol-stress animals.

Corticosterone Assay

Serum corticosterone levels were determined from the tail blood samples acquired during the electrophysiological recording period and analyzed using a commercial radioimmunoassay kit (MP Biomedials, Orangeburg, NY; catalog no. 07-0120103) according to the manufacturer’s instructions.

Electrophysiology

All electrophysiology was performed in the offspring of dams from the ethanol, paired, and ad libitum groups when they were between PND30 and PND35. It is important to note again here that animals in the ethanol and paired groups were...
only exposed to their respective conditions in utero, and that all animals were on equivalent diets from birth until their use in these experiments. All animals were anesthetized with urethane (1.5 mg/kg) and placed in a stereotaxic apparatus (Kopf Instruments). Rectal temperature was maintained at 37°C ± 1°C with a grounded homeothermic temperature control unit (Harvard Instruments, MA). Two ground screws were inserted in the skull anterior to bregma and at lambda to ground the recording and stimulating electrodes, respectively. A 125-μm stainless steel recording electrode was directed through a trephine hole into the CA1 (3.0 mm posterior, 3.0 mm lateral to bregma). Additionally, a 125-μm monopolar stimulating electrode was directed through the same trephine hole to stimulate the Schaffer-collateral/commissural pathway. The final depth of the stimulating and recording electrodes was determined by adjusting both electrodes to yield a maximal field excitatory postsynaptic potential (EPSP). The final stimulation intensity was adjusted to elicit an EPSP that was 60% of the maximal EPSP size, within a 1–2 mA range.

Paired-pulse facilitation was assessed by administering two pulses at both 50- and 100-μs interpulse intervals. Following the presentation of the paired-pulse stimuli, baseline evoked responses were acquired using single pulse stimuli (120 μs) delivered every 15 s. After acquiring a stable baseline for at least 15 min, LFS (900 pulses at 3 Hz) was administered using the same pulse width. Following LFS, baseline stimulation was again administered for 2 h to assess the long-term effects of the LFS (followed by paired-pulse facilitation stimulation). All electrical signals were amplified and filtered (1 Hz and 3 kHz using a differential amplifier (Getting Instruments, San Diego, CA) and then digitized at 5 kHz before being stored on a PC using custom-written software (Lee Campbell; Getting Instruments) and National Instruments data acquisition hardware.

### Data and Statistical Analysis

The initial phase of the EPSP slope (10–80%) was assessed at 115- to 210-min post-LFS to assess LTD expression. All LTD data are presented as the mean percent EPSP change from baseline ± SEM. Either planned comparisons or analysis of variance (ANOVA) was performed on data and, where appropriate, the ANOVA was followed by Newman-Keuls post hoc analyses. All analyses were performed using Statistica software (Statsoft, Tulsa, OK), with statistical significance set at P < 0.05.

### RESULTS

#### Effects of Sex and Diet on the Development of the Offspring

The average ethanol intake for the ethanol dams throughout gestation was 11.83 ± 0.27 g/kg body wt/day and the average BEC on GD15 was 192 ± 21 mg/dl, a value similar to that observed in previous studies from our laboratory (Christie et al., 2005; Redila et al., 2006). The developmental data for ethanol, paired, and ad libitum females and offspring is presented in Table 1. As we have reported previously (Christie et al., 2005), the ethanol diet delays parturition by 1.023 days on average compared to ad libitum animals. The pairfed animals gave birth 0.45 days later than the ad libitum animals on average. Prenatal diet did not affect postnatal weight, as there was no significant difference in weight between PND2 and PND22 for male ethanol, pairfed, and ad libitum animals (F(2,25) = 1.96, P = 0.16) or female ethanol, pairfed, and ad libitum animals (F(2,25) = 0.61, P = 0.54). Furthermore, between PND2 and PND22 there were no weight differences between males and females animals (F(2,50) = 0.24, P = 0.78), regardless of prenatal diet. However, between PND30 and PND35, male offspring did weigh more than female offspring (F(1,111) = 38.75, P = 0.00), although at this age both ethanol males and females weighed less pairfed than their pairfed and ad libitum counterparts (F(2,11) = 6.56, P = 0.00).

#### Effects of Acute Stress on Corticosterone Levels

Previous studies have found that ethanol-exposed animals are not hyper-responsive to stress at PND39, compared with control animals, although they do still exhibit a stress response (Weinberg and Gallo, 1982). Therefore, it was of interest to compare corticosterone levels between ethanol, pairfed, and ad libitum animals to determine whether ethanol animals between PND30 and PND35 exhibit a similar stress response as ad libi-

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**Table 1. Developmental Data for Ethanol, Pairfed and Ad libitum Dams and Offspring from Birth to PND35**

| Treatment | 
| --- | --- | --- |
| | Ethanol | Paired | Ad libitum |
| Pregnancy outcome variables | | | |
| Number of pregnant dams | 8 | 10 | 10 |
| Gestation length (days) | 22.25 ± 0.25 | 21.8 ± 0.133 | 21.48 ± 0.15 |
| Number of male pups | 6.62 ± 0.53 | 7.1 ± 1.10 | 7.40 ± 0.80 |
| Number of female pups | 7.57 ± 0.80 | 6.9 ± 0.87 | 7.90 ± 0.92 |
| Male pup weight (g) | | | |
| PND2 | 5.03 ± 0.10 | 6.57 ± 1.39 | 6.56 ± 1.42 |
| PND8 | 13.57 ± 0.47 | 17.66 ± 3.75 | 17.77 ± 4.70 |
| PND15 | 27.54 ± 4.03 | 33.11 ± 6.79 | 32.52 ± 6.66 |
| PND22 | 42.28 ± 5.98 | 55.21 ± 11.35 | 49.77 ± 7.71 |
| Female pup weight (g) | | | |
| PND2 | 5.92 ± 1.26 | 5.82 ± 0.80 | 7.04 ± 0.77 |
| PND8 | 13.73 ± 3.56 | 14.80 ± 2.45 | 16.19 ± 3.35 |
| PND15 | 26.57 ± 4.32 | 29.29 ± 4.35 | 27.15 ± 6.82 |
| PND22 | 41.15 ± 9.27 | 46.48 ± 7.87 | 46.28 ± 28 |
| PND 30–35 weight (g) | | | |
| Male | 103.00 ± 3.08 | 112.57 ± 3.56 | 116.26 ± 7.62 |
| Female | 78.38 ± 1.78 | 88.04 ± 3.99 | 98.72 ± 3.45 |

*Significantly longer than Paired and Ad libitum.

*Significantly larger than females on corresponding PND.
Effects of Ethanol, Diet, and Sex on Paired Pulse Facilitation

Paired-pulse facilitation was examined to determine whether stress or prenatal diet altered presynaptic transmitter release in the CA1 region (Fig. 2). In male animals, there was a significant main effect of interpulse interval \( (F_{(1,108)} = 131.01, P = 0.00) \) with greater PPF at 50 ms than 100 ms \( (P = 0.00) \). Comparisons were then made in female animals revealing a significant main effect of interpulse interval \( (F_{(1,94)} = 126.41, P = 0.00) \); a 50 ms IPI elicited greater PPF than at 100 ms \( (P = 0.00) \). Importantly, there was no effect of prenatal diet on the amount of PPF elicited in males \( (F_{(2,108)} = 1.18, P = 0.31) \) or females \( (F_{(2,94)} = 3.01, P = 0.06) \), indicating that neither PNEE nor prenatal food deprivation alters presynaptic neurotransmitter release.

Effects of Stress on LTD in Male and Female Animals

To determine whether male and female animals differed in their capacity to exhibit LTD in the CA1 region, we examined the effects of LFS in both the naïve animals and in the animals that were exposed to acute stress (platform isolation) for 30 min. The change in EPSP slope at 120 min post-LFS was different between males and females, as shown in Figure 3A. Specifically, naïve ad libitum males did not exhibit LTD \((-3.16\% \pm 3.22\%; t_{(8)} = -0.98, P = 0.36)\), while significant LTD was observed in stress ad libitum males \((-29.61\% \pm 8.44\%; t_{(10)} = -3.51, P = 0.01)\), similar to previous studies (Xu et al., 1998; Xiong et al., 2003, 2004). On the other hand, significant LTD was observed in naïve ad libitum females \((-18.33\% \pm 5.99\%; t_{(12)} = -3.06, P = 0.01)\) but not in the stress ad libitum females \((2.64\% \pm 4.43\%; t_{(9)} = 0.60, P = 0.57)\); Fig. 3B). The amount of LTD in the naïve ad libitum females was not significantly different from that observed in the stress ad libitum males \((t_{(20)} = 1.12, P = 0.23)\).

Effects of Prenatal Food Deprivation on LTD in Male and Female Animals

Identical experiments to those conducted in the offspring of the ad libitum dams were also conducted in the offspring of the paired dams. In contrast to the results obtained in the ad libitum offspring, the male paired offspring showed significant LTD in the absence of stress \((naive: -23.77\% \pm 4.44\%; t_{(12)} = -5.35, P = 0.00)\). In addition, a significant level of LTD was observed in the male stress paired animals \((-15.73\% \pm 5.28\%; t_{(9)} = -2.98, P = 0.02)\). The amount of LTD observed at 120 min post-LFS in the two male paired offspring conditions was not significantly different \((t_{(13)} = -1.17, P = 0.26)\); Fig. 4A).

A similar pattern was observed in the female paired offspring. Significant LTD was observed in female paired naïve offspring \((-12.57\% \pm 5.57\%; t_{(13)} = -2.25, P = 0.04)\) as well as in the female paired stress animals \((-19.48\% \pm 4.96\%; t_{(9)} = -3.93, P = 0.00)\). Once again, the amount of

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LTD observed in both female pairfed offspring groups was not significantly different \( (t_{20} = 0.88, P = 0.39; \text{Fig. 4B}) \). In addition, the amount of depression observed in both pairfed male and female offspring was not significantly different, regardless of the stress condition \( (F_{1,39} = 2.03, P = 0.16) \).

Effects of PNEE on LTD in Male and Female Animals

To determine whether prenatal exposure to ethanol had deleterious effects on synaptic plasticity in the hippocampus, we examined LTD in male and female offspring from ethanol dams. Male ethanol offspring did not exhibit significant LTD in the absence of stress \( (t_{8.01} = 7.51\%; t_{10} = 1.06, P = 0.31) \) but, similar to ad libitum offspring, did exhibit significant LTD in the stress condition \( (t_{9} = 2.38, P = 0.04) \), even though the magnitude of the change in EPSP slope in the naïve and stress ethanol male offspring was not significantly different \( (t_{17} = 0.18, P = 0.86; \text{Fig. 5A}) \).

Although not significant, there was a trend for naïve ethanol female offspring to exhibit LTD \( (-15.57\% \pm 7.10\%; t_{10} = -2.19, P = 0.06) \). However, similar to stress ad libitum female offspring, significant LTD was not induced following stress \( (-4.21\% \pm 5.88\%; t_{7} = -0.72, P = 0.50) \). Again, there was no difference in the magnitude of change in the EPSP slope in the stress and naïve ethanol female offspring \( (t_{15} = -1.15, P = 0.27; \text{Fig. 5B}) \).

Effect of Acute Stress on LTD Across Prenatal Diets

The results thus far indicate that acute stress does not uniformly affect CA1 LTD in males and females. As well, the capacity for LTD in CA1 is enhanced following prenatal food deprivation, because significant LTD was observed in naïve

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pairfed offspring. Interestingly, PNEE does not seem to have deleterious effects on CA1 LTD in males because ethanol male offspring exhibit LTD only following acute stress, similar to control males. On the other hand, ethanol female offspring do not exhibit LTD in the absence of stress, in contrast to naive ad libitum female offspring. Taken together, these results suggest that the capacity for LTD in males is unaffected by PNEE but enhanced by prenatal nutritional deprivation. In females, PNEE reduces LTD in naive ethanol female offspring but enhances LTD in pairfed female offspring.

To determine whether the magnitude of LTD in pairfed and ethanol offspring was similar to ad libitum animal offspring, comparisons were made between male offspring, and between female offspring, from each group. In addition, the animals from each group were further subdivided into either stress or nonstress (naive) conditions (Fig. 6). A one-way ANOVA revealed a main effect of diet in male naive offspring ($F_{(2,27)} = 3.94, P = 0.03$), and post hoc analyses revealed that male pairfed offspring had significantly more LTD ($-23.77 \pm 4.44$) than male ad libitum offspring ($-3.16 \pm 3.22, P = 0.03$); there was a trend toward greater LTD in pairfed male offspring than in ethanol male offspring ($-8.01 \pm 7.50, P = 0.06$). Surprisingly, there was also a trend toward a significant relationship of prenatal diet and LTD in stress male offspring ($F_{(2.25)} = 2.61, P = 0.09$), with less depression in ethanol male offspring than controls ($P = 0.09$).

Female naive offspring exhibited similar amounts of LTD regardless of prenatal diet ($F_{(2.32)} = 0.23, P = 0.79$), but there was a main effect of prenatal diet in females exposed to acute stress ($F_{(2.22)} = 5.44, P = 0.01$). Post hoc analyses did not

[FIGURE 3. Long-term depression in male and female ad libitum animals. (A) Three hertz LFS does not induce LTD in male ad libitum naive ($n = 8$) animals, but does result in LTD in male ad libitum animals exposed to an acute stress (elevated platform) for 30 min ($n = 10$). Note that despite the persistent downward trend in the stress group, the final 20 min is stable. (B) Female ad libitum naive animals ($n = 13$) exhibits LTD following 3-Hz stimulation, whereas female ad libitum stress animals ($n = 9$) do not show LTD. Scale bar is 1 mV by 5 μs.]

[FIGURE 4. Long-term depression in male and female pairfed animals. (A) LTD can be induced in male pairfed naive ($n = 11$) animals and there is a depression of EPSP slope in male pairfed stress ($n = 9$) animals. (B) Stress was required for LTD in female pairfed animals ($n = 9$), as there was a slight depression of EPSP slope in female pairfed naive (FPNS, $n = 13$) animals, although this did not reach significance. Traces correspond to immediately prior to LFS (1) and 2 h after LFS (2). Scale bar is 1 mV by 5 μs.]
reveal significant LTD in ad libitum female offspring, while LTD was only observed in stress ad libitum male offspring. In contrast, food deprivation in utero (paired animals) established a more permissive environment for the induction of LTD in both male and female offspring. Furthermore, LTD could be observed in both sexes following prenatal food deprivation regardless of whether they were exposed to stress or not. Surprisingly, PNEE did not affect the capacity for LTD in naive female offspring, but there was a trend toward reduced LTD in stress ethanol offspring compared to stress ad libitum male offspring. The capacity for LTD across groups was not associated with changes in presynaptic release mechanisms, as paired-pulse facilitation was not significantly different across sex or prenatal diet.

Effects of Stress on LTD in Male and Female Animals

Previous studies have shown that exposure to stress can facilitate the induction of LTD in male animals (Xu et al., 1998; Xiong et al., 2003, 2004), which was replicated in the current study. In contrast to the ad libitum male offspring, female offspring reliably expressed LTD in the absence, but not in the presence of stress. This is the first study to investigate the effects of stress on LTD in young female animals in vivo, and as such, it is important to acknowledge that the observed differences in synaptic plasticity between males and females might reflect differences in estrogen levels (Good et al., 1999; Desmond et al., 2000; Zamani et al., 2000; Day and Good, 2005; Shiroma et al., 2005). These previous studies provide evidence that estradiol can enhance LTP and reduce LTD. Since LTD was enhanced in control females with the current study, it does not seem likely that estradiol influenced synaptic plasticity in these prepubescent females. However, we did not directly determine estradiol levels in the animals used in this study, and future studies will need to investigate whether there is any impact of sex hormones on synaptic plasticity at PND30–35, because this is within the range for the onset of puberty (Gabriel et al., 1992). It is noteworthy that although the stress response appears equivalent in male and female animals at PND39 (Weinberg and Gallo, 1982), our results indicate that sex differences do exist in both the physical response to stress and the effect of stress on synaptic plasticity as early as PND30. Acute stress differentially affects learning and memory in male and female animals (Beiko et al., 2004; Conrad et al., 2004; Shors, 2004; Hodes and Shors, 2005), with impairments more common in males. As glucocorticoid receptors (GRs) are active in response to stress (Reul and de Kloet, 1985; Reul et al., 1987; Spencer et al., 1993), these findings suggest that acute stress may not lead to uniform GR occupancy in males and females. In adult females, GRs have greater $B_{\text{max}}$ and higher $K_d$ values than males, which is not affected by prenatal food deprivation or PNEE; GRs in females have similar properties and are likewise unaffected by prenatal diet (Weinberg and Petersen, 1991). Less corticosterone is required for the activation of mineralocorticoid receptors (MRs) than GRs (Reul

DISCUSSION

There were a number of important and interesting findings in the present study. First, the capacity for LTD expression in vivo is different in male and female animals, even when they are tested prior to puberty, as was the case in this study. The
and de Kloet, 1985), and the receptors have opposing effects on synaptic plasticity, such that MR activity enhances LTP (Pavlides et al., 1996) while GR activity enhances LTD (Xu et al., 1998). Therefore, in order to depress synaptic plasticity, optimal levels of corticosterone are required in order to activate GRs. However, if too little corticosterone is produced in response to stress, predominant MR activation might result that would enhance synaptic plasticity. Within the current study, naive ad libitum and ethanol female offspring exhibited robust LTD, which was blocked by acute stress, suggesting that the acute stress may have occupied MRs, effectively shifting the induction threshold for LTP. It is also possible that the threshold for LTD induction is different between males and females (Brennan et al., 2007) and may contribute to the sex differences in LTD observed in this study. How stress affects CA1 LTD in females has yet to be fully examined, but it is clear from this study that there are basic sex differences in the capacity of male and females to exhibit synaptic plasticity in the CA1 region that are apparent as early as PND30–35.

Effects of Prenatal Food Deprivation on Synaptic Plasticity

The prenatal diets employed in this study were designed to provide adequate nutrition to dams, regardless of the amount of food diet consumed (Weinberg, 1985), although pairfed and ethanol dams still consume fewer proteins and calories overall compared with ad libitum dams (Weinberg, 1985). This prenatal food deprivation did alter CA1 plasticity, as significant LTD was observed in pairfed male and female offspring, regardless

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Effects of PNEE on Synaptic Plasticity

The direct effects of ethanol on LTD have yielded mixed results, with one report showing LTD blocked while the other shows it to be enhanced (Hendricson et al., 2002; Thinschmidt et al., 2003). The current study is the first to investigate the relationship between PNEE and LTD and found that prenatal exposure to this teratogen does not produce a long-lasting impairment in the ability of animals to express LTD. Our initial comparisons indicated that stress was required for LTD in ethanol male offspring while there was a trend toward blocked LTD in ethanol female offspring following acute stress. These findings were similar to the respective ad libitum control offspring, and so comparisons were made across prenatal diets. These revealed that LTD was not significantly affected by prenatal ethanol exposure. Specifically, there was no significant difference in the amount of LTD observed between stress ad libitum and ethanol male offspring or between naive ad libitum and ethanol male offspring. Furthermore, there was no significant difference between the amount of LTD in naive ad libitum and ethanol female offspring, nor between stress ad libitum and ethanol male offspring. Therefore, in contrast to the results obtained in paired offspring, the effect of stress on CA1 LTD in males and females is preserved following prenatal ethanol exposure.

Previous studies have indicated that PNEE attenuates hippocampal LTP in adult animals (Swartzwelder et al., 1988; Sutherland et al., 1997; Christie et al., 2005) although LTP does appear to be altered in young males (Krahl et al., 1999). A heightened stress response is also not apparent in young animals (Weinberg and Gallo, 1982). Taken together, this suggests that the adolescent brain might not be as susceptible to the deleterious effects of prenatal ethanol exposure, but that these impairments become apparent with age. The results of the current study support this notion since basal and stress cort levels, as well as the amount of LTD in ethanol males and female offspring was not significantly different from their ad libitum counterparts. The functional integrity of GRs and MRs in the hippocampus are not affected by PNEE (Weinberg and Petersen, 1991), which may account for the LTD in the male ethanol stress condition, since it presumably relies upon functional GRs in the hippocampus (Xu et al., 1998). Furthermore, the acute stress may have preferentially occupied MRs in ethanol female offspring, which would account for the reduced LTD observed following acute stress.

It is curious that significant LTD was not observed in naive and stress ethanol male offspring and female offspring, as was found in paired animals. Ethanol dams have elevated corticosterone levels, when compared with both paired and ad libitum dams in response to acute stress (Weinberg and Gallo, 1982), suggesting that the ethanol diet can alter hypothalamic–pituitary–adrenal (HPA) activity. Ethanol can also directly stimulate the HPA axis (Rivier, 1993, 1996; Ogilvie and Rivier, 1996; Rivier and Lee, 1996), which might cause enhanced HPA activity in response to stress, compounding the elevated corticosterone levels in ethanol dams following acute stress. However, the actions of ethanol on the HPA axis might not be “stressful,” but instead simply activates the HPA axis. Indeed, animals with PNEE have elevated adrenocorticotropic hormone levels when exposed to a stressor but not following infusion of CRH (Lee et al., 2000), suggesting that artificial stimulation of the HPA axis might not produce the same effect as actually experiencing stress. Therefore, the ethanol exposed dams might have elevated corticosterone because of the stimulatory effects of ethanol on the HPA axis, but the paired dams experienced the stress of food deprivation. Thus, LTD was expressed in both naive and stress paired offspring due to the mild stress experienced by the paired dam throughout gestation. This hypothesis is supported by the finding that prenatal stress can enhance the induction of LTD in offspring (Yang et al., 2006). In contrast to ethanol offspring, paired offspring do not always exhibit elevated corticosterone levels in response to stress (Weinberg and Gallo, 1982; Weinberg, 1988, 1992), and LTP in paired animals is not different from controls (Sutherland et al., 1997; Savage et al., 2002; Christie et al., 2005). From the present findings, it is apparent that LTD is more sensitive to prenatal food deprivation than it is to prenatal ethanol exposure, even though PNEE is known to adversely impact hippocampal LTP (Sutherland et al., 1997; Savage et al., 2002; Christie et al., 2005).

### SUMMARY

Young male and female animals possess different capacities to exhibit LFS-induced LTD in vivo, with females being more likely to exhibit LTD than males. Acute stress has opposing...
effects on synaptic plasticity in each sex, enabling the induction of LTD in males, but impairing it in females. Although normally a control group for prenatal ethanol exposure, the paired offspring all showed LTD, indicating that this manipulation can affect synaptic plasticity. More importantly, this LTD could be induced in the paired offspring, irrespective of whether they were exposed to acute stress prior to experimentation or not. This would indicate that the prenatal stress associated with restricted food intake in utero is a main factor in influencing the capacity for LTD in these animals. Surprisingly, this is not the case for LTP, which does not appear to be inhibited by the paired diet (Sutherland et al., 1997; Christie et al., 2005). In contrast to our expectations, PNEE did not significantly impair LTD in young males or females, although there was a trend for it to reduce LTD in naive male offspring, as compared to ad libitum male offspring. Further research will be needed to determine what mechanisms associated with LTD and not LTP are affected by prenatal dietary restriction.

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