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CHRONIC PRENATAL ETHANOL EXPOSURE IMPAIRS CONDITIONED RESPONDING AND ENHANCES GABA RELEASE IN THE HIPPOCAMPUS OF THE ADULT GUINEA PIG

M.L. Hayward[1], A.E. Martin[2], J.F. Brien[1,3], H.C. Dringenberg[1,2,3], M.C. Olmstead[1,2,3] and J.N. Reynolds[1,3]

Departments of Pharmacology & Toxicology, and Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6

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Author for Correspondence: Dr. J.N. Reynolds
Pharmacology & Toxicology
Queen's University
Kingston, Ontario, Canada, K7L 3N6

Tel: (613) 533-6946
Fax: (613) 533-6412
email: jnr@post.queensu.ca

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List of Abbreviations:

ANOVA – analysis of variance
BGS – brain growth spurt
CPEE – chronic prenatal ethanol exposure
CRF – continuous reinforcement
FAS – fetal alcohol syndrome
FR – fixed ratio
GABA – γ-aminobutyric acid
GD – gestational day
HPLC – high-performance liquid chromatography
LTP – long-term potentiation
MT – magazine training
NMDA – N-methyl-D-aspartic acid
PD – postnatal day

Abstract

In this study, we assessed the effects of chronic prenatal ethanol exposure (CPEE) on spatial navigation in the water maze, conditioned responding using food-reinforced lever pressing, and amino acid neurotransmitter release from the hippocampus of the adult guinea pig. Pregnant guinea pigs were treated with ethanol (3g/kg maternal body weight/day), isocaloric-sucrose/ pair-feeding, or water throughout gestation. Adult offspring were trained in 2-lever operant chambers, with one lever designated as the rewarded lever, to respond for sucrose pellets. There were no group differences in response acquisition or lever discrimination on a fixed-ratio 1 (FR-1) schedule. During extinction sessions, CPEE offspring maintained higher levels of responding on the previously reinforced lever, suggesting that CPEE increases perseveration and/or impairs response inhibition, but does not affect operant responding for an appetitive reinforcer or the ability to discriminate rewarding from non-rewarding stimuli. In contrast, for the maternal ethanol regimen employed in this study, there was no effect of CPEE on performance in the water maze. CPEE did not alter electrically-evoked glutamate or GABA release from hippocampal brain slices. However, when slices were tested after delivery of a tetanizing stimulation (five 5-s trains at 100Hz), post-tetanic potentiation of electrically-stimulated GABA release was greater in hippocampal slices obtained from CPEE offspring, whereas post-tetanic potentiation of electrically-stimulated glutamate release was unaffected. These data suggest that conditioned learning is a sensitive behavioural measure of CPEE-induced brain injury. Increased activity-dependent potentiation of GABA release in the hippocampus may contribute to alterations in synaptic plasticity observed in CPEE offspring.

Chronic prenatal ethanol exposure (CPEE), via maternal ethanol administration, can produce a spectrum of neurobehavioural deficits, including the fetal alcohol syndrome (FAS; Abel, 1995; Clarren and Smith, 1978; Jones and Smith, 1973). In particular, CPEE can induce brain injury in offspring that manifests as life-long learning and cognitive deficits.

Animal models have been used to gain insight into the neurobehavioural deficits that result from CPEE. In particular, the effects of CPEE on learning and memory have received a great deal of attention. In the rat (Blanchard et al., 1987; Reyes et al., 1989; Matthews and Simson, 1998) and the guinea pig (Richardson et al, 2002; Iqbal et al., 2003), CPEE impairs spatial learning in postnatal offspring. Similarly, in tests of operant learning, CPEE has been reported to produce impairments in performance (Driscoll et al., 1980; Riley et al., 1980), the magnitude of which may be dependent on the complexity of the task (Mihalick et al., 2001).

Among brain regions adversely affected by CPEE, the hippocampus appears to be especially vulnerable (West and Pierce, 1986). Brain imaging studies have revealed gross hippocampal dysmorphology in children with FAS (Mattson et al., 2001; Riikonen et al., 1999), and animal models of the disorder have demonstrated selective loss of CA1 pyramidal cells (Gibson et al., 2000), altered mossy fiber and pyramidal cell arrangement (Sakata-Haga et al., 2003), and decreased dendritic spine density (Ferrer et al., 1988) in the hippocampus. CPEE induces deficits in spatial learning tasks that are sensitive indicators of hippocampal injury (Richardson et al., 2002; Berman and Hannigan, 2000), and impairs synaptic plasticity in the CA1 region of the hippocampus (Richardson et al., 2002) and in the perforant path-dentate gyrus granule cell pathway (Sutherland et al., 1997).

Synaptic plasticity arises from temporally coordinated changes in both presynaptic neurotransmitter release and postsynaptic receptor function that cause long-lasting changes in the

efficiency of synaptic communication. Direct imaging has confirmed that presynaptic function is enhanced during long-term potentiation (LTP) in the CA1 region of mammalian hippocampal slices (Zakharenko et al. 2001). At present, however, only a few studies have been conducted to investigate the potential role of neurotransmitter release in CPEE-induced alterations in synaptic plasticity (Savage et al., 1998, 2002), and none have examined endogenous glutamate and GABA release simultaneously. The purpose of this study was to determine the effects of CPEE, via daily maternal administration of ethanol, on electrically stimulated amino acid neurotransmitter release in the hippocampus of the adult guinea pig. In addition, we compared the effects of CPEE on performance in two different learning paradigms, spatial navigation in the water maze and conditioned responding in a lever pressing task, which may be adversely affected by CPEE.

Methods

Experimental Animals

All experimental animals were cared for according to the principles and guidelines established by the Canadian Council on Animal Care. The experimental protocol was approved by the Queen's University Animal Care Committee. An established breeding procedure (Elvidge, 1972) was used to mate nulliparous female Dunkin-Hartley-strain guinea pigs (500-600 g body weight; Charles River Canada, St. Constant, QC) with male guinea pigs of the same strain. The last day of full vaginal membrane opening was defined as gestational day (GD) 0 (term, about GD 68). On GD 2, pregnant animals were housed individually in large, stainless steel cages. Lighting was on a 12-hour light/dark cycle, with lights on at 0700. Animals were monitored daily for general health, body weight, food consumption, and vaginal membrane status. On the day of parturition (postnatal day (PD) 0), litters were placed in large plastic bins with wood chip bedding. Offspring were weighed and monitored for general health daily starting at PD 1, and were weaned and separated by gender on PD 17.

Chronic Treatment Regimens

Pregnant guinea pigs were assigned to one of three treatment groups: (1) 3 g ethanol/kg maternal body weight/day with *ad libitum* access to pellet food and water; (2) isocaloric, isovolumetric sucrose with pair-feeding and *ad libitum* access to water; or (3) isovolumetric water with *ad libitum* access to food and water. Treatment solutions were 30% ethanol (v/v, in tap water), 42% sucrose (w/v in tap water) or plain tap water, respectively. The pair-feeding regimen involved pairing each sucrose-treated animal with an ethanol-treated animal; each day, the sucrose-treated guinea pig received food equal to the amount eaten by its ethanol-pair on the same gestational day. Daily treatments were given by intubation into the oral cavity from GD 2

to GD 67. All treatments were administered in two equally divided doses two hours apart, beginning between 0800 h and 0930 h each day. At GD 57, a 200-µl sample of blood was taken from an ear blood vessel of each ethanol-treated animal 1 h after the second divided dose, and a gas-liquid chromatographic method (Steenaart et al., 1985) was used to determine blood ethanol concentration. Blood also was taken from the sucrose-treated and water-treated guinea pigs to control for the stress induced by the procedure.

Morris Water Maze

The Morris water maze consisted of a circular pool 180 cm in diameter. Water, made opaque by the addition of a nontoxic white paint, was used to fill the maze. A hidden escape platform, 30 cm in diameter, was located in the centre of one of the quadrants of the pool 2 cm below the surface of the water. The water temperature of the maze was maintained at 21-22°C.

Beginning at approximately PD 50 (range, PD 45-55), male and female offspring were trained in the water maze for seven consecutive days (acquisition phase). Four trials were administered on each day, given in two blocks of two trials with a 5-min rest period between the blocks. For each trial, the animal was placed into one quadrant of the pool, facing the wall. Animals were allowed a maximum of 45 s to swim to the hidden escape platform; animals that failed to locate and mount the platform within this time limit were retrieved from the pool and placed on the platform. Once on the platform the animal was allowed to remain on it for 15 s. The time to reach the hidden platform was recorded for each trial. Four different start positions, one from each quadrant of the pool, were used in a randomized order for the four trials administered each day. All trials were videotaped using a camera mounted above the pool.

Seven days after the last day of training, a probe trial was administered to test retention performance. This involved removing the escape platform from the pool, and allowing the

animal to explore the pool for 60 seconds. The time spent in the quadrant where the platform was located during training was recorded.

Conditioned Responding

Subjects

The subjects were male and female guinea pig offspring from mothers in one of the three chronic maternal treatment regimens: ethanol, isocaloric-sucrose/pair-fed, and water treatments. Where possible, subjects were chosen such that there was a male and female animal from each litter in each treatment group. When litters did not contain a male and female, either two offspring of the same sex were chosen or a single member was included, in order to make the male:female ratio as uniform as possible. The number of subjects in each group were: 4 females and 7 males from the ethanol treatment group (ethanol, n = 11 offspring obtained from 8 litters), 7 females and 5 males from the isocaloric-sucrose/pair-fed treatment group (sucrose, n = 12 offspring obtained from 8 litters), and 6 females and 6 males from the water treatment group (water, n = 12 offspring obtained from 8 litters). Training in the operant paradigm began on average at about PD 63 (S.E.M. = $\pm$0.40).

Animals were kept on a 12-hr light/dark cycle, with lights on at 0700. Prior to the start of operant training, subjects were given *ad libitum* access to both food and water; during operant training, animals were food deprived for approximately 7 h before each session. Subjects had *ad libitum* access to water at all times. On the day before training began, subjects were exposed to sucrose by adding approximately 16 g of sucrose pellets to their food.

Apparatus

Training occurred in operant boxes (26.5 cm long x 22.0 cm wide x 20.0 cm high), with each animal housed individually in a sound-attenuating chamber. The operant chamber was fitted with two retractable levers (4-cm wide), positioned on one wall, 13 cm apart and 5 cm

above the plexiglass floor. A food magazine was located between the two levers. Sucrose pellets (45 mg, Bio-Serv, NJ, USA) were delivered to the magazine via a pellet dispenser (Med Associates, Vermont, USA). Pellet delivery was signaled by a dim light located 4 cm above the magazine, and infrared sensors detected pellet collection. An IBM-type computer controlled the operant equipment and was used to collect data (software written in house).

Habituation

Habituation to the operant chambers occurred for 30 min per day. During habituation sessions, animals were placed in the operant chambers. Levers remained retracted for these sessions and 20 sucrose pellets were delivered at random intervals. Subjects were required to make a minimum of 20 nosepokes to the magazine in at least one of two sessions in order to progress to the next stage of training. Subjects that did not reach criterion after 8 days were removed from training. Five females and 6 males from the water group (n = 11), 7 females and 2 males from the sucrose group (n = 9), and all animals from the ethanol group (n = 11) completed this phase of training.

Continuous Reinforcement (CRF) Training

During CRF training, both levers were extended into the chamber. One lever was designated the reinforced lever, and one pellet was delivered for each press on this lever. The depression of the other lever had no consequences. Levers were counterbalanced such that half the animals in each group had the right lever reinforced, and the other half the left lever reinforced. Subjects completed one session per day. A maximum of 50 sucrose pellets could be obtained in a 30-min session, and if the maximum was reached before 30 min, the session was terminated. The number of reinforced and non-reinforced lever presses was recorded. To complete the CRF schedule, an animal had to receive at least 45 rewards and make at least 75% of their responses on the reinforced lever on two consecutive days. Subjects that did not reach

this criterion after 20 days were removed from training. Twenty days is approximately two standard deviations higher than the average number of days required by the subjects that successfully reached criterion. Four females and 5 males in the water group (n = 9), 4 females and 2 males in the sucrose group (n = 6), and 3 females and 5 males in the ethanol group (n = 8) completed this phase of training.

Increasing Fixed Ratio (FR) Schedules

Subjects reaching the CRF schedule criterion were moved onto a FR schedule. Session parameters and lever designations were the same as those used during the CRF training. Criterion to complete a given FR schedule was two consecutive days in which the animal received at least 45 sucrose pellets and made at least 75% of their responses on the reinforced lever. The schedule increased by increments of two (eg. FR-3, FR-5, FR-7) to a maximum of FR-33. Subjects that did not reach criterion for a given schedule after 8 days were removed from training. Eight days is approximately two standard deviations higher than the average number of days taken for the remaining subjects to reach criterion at each FR step. Three animals (2 from the water group and 1 from the ethanol group) completed FR-33. Data for the first two-thirds of subjects not reaching criterion were organized into six blocks, as shown in Table 1. Analyses by likelihood ratio χ^2 tests of the drop-out rates revealed no differences between treatment groups ($p > 0.05$). Therefore, the remaining subjects, 2 from the water group, 4 from the sucrose group, and 5 from the ethanol group, were also moved from FR training to extinction sessions.

Extinction

All subjects tested under increasing FR schedules were tested in extinction. Animals completed 3 consecutive days of CRF sessions prior to extinction sessions. During extinction, there were no consequences for pressing either lever. Responses on the previously reinforced and non-reinforced levers were recorded over four 30-min sessions.

Hippocampal Slice Preparation

Between PD 70 and PD 100, male and female guinea pig offspring were weighed, anaesthetized with halothane and euthanized by decapitation. The brain was quickly excised, weighed, and placed in ice-cold buffered saline (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 10 mM glucose, pH 7.4, saturated with 95% O_2/5% CO_2). A vibrating microtome (TSE Systems, Hamburg, Germany) was used to cut 400-μm-thick coronal slices containing dorsal hippocampus. In a small petri dish filled with ice-cold, oxygenated, buffered saline, the hippocampal tissue was carefully isolated using fine-tipped brushes, and the left and right hippocampi in each slice were separated by scalpel cut. The hippocampal slices were randomly separated into two sets, and used for determination of either the input-output relationship between electrical stimulation and amino acid neurotransmitter release, or the effects of tetanic stimulation on electrically stimulated amino acid neurotransmitter release.

Basal and Electrically Stimulated Amino Acid Neurotransmitter Efflux

A superfusion system (BSC-440, Warner Instruments, Hamden, CT) was used to determine basal and electrically stimulated release of glutamate and GABA in the hippocampus. The superfusion system was coupled to a bipolar pulse generator (BTS-404, Warner Instruments, Hamden, CT) for electrical stimulation of hippocampal slices in the four perfusion chambers. A single hippocampal slice was placed into each chamber of the apparatus, which then was submerged in a 37°C water bath, and slices were perfused throughout the experiment with oxygenated, buffered saline at a flow rate of 0.8 ml/min. After a 2-h equilibration period, two 3-min perfusate fractions were collected and used to determine baseline neurotransmitter release.

In half of the chambers, sequential, increasing stimulation strengths of 25, 50, 60 and 75 mA (20 Hz, 5-ms pulse width, 3-min duration) were delivered. For each stimulus applied, three consecutive 3-min perfusate fractions were used to capture the evoked neurotransmitter release. Collection of the first fraction began simultaneously with initiation of electrical stimulation, and delivery of the next stimulus began following collection of the third fraction (interpulse interval of 6 minutes). Pilot studies showed that this time interval was sufficient for the release of amino acid neurotransmitter substances to return to baseline (data not shown). The remaining chambers were used for the tetanus-induced potentiation paradigm. A control, submaximal stimulus of 50 mA (20 Hz, 5-ms pulse width, 3-min duration) was given, and three 3-min fractions were collected as described for the input-output paradigm. Following completion of collection of the third fraction, a tetanizing stimulus was applied to the chamber (five trains of stimulation at 99 mA, 100 Hz, 2-ms pulse width, interstimulus interval of 5 s). At 5 and 15 min after the end of the tetanus, a 50 mA submaximal stimulation identical to the pre-tetanus control stimulus was administered, and three 3-min fractions were collected as previously described. Following experimentation, the hippocampal slice in each chamber was digested in 1 N NaOH, and the amount of protein was determined using a modified Lowry assay (Lowry et al., 1951).

Amino Acid Analysis

Glutamate and GABA in the perfusate fractions were quantified using reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection of their thiol-substituted isoindole derivatives (Butters et al., 2000). The individual hippocampal slices were considered viable and included in data analysis if the amount of neurotransmitter release in the perfusate was stable over the two basal fractions (<30% variation) and the 50 mA stimulation increased neurotransmitter amount by at least 1.5-fold above basal value.

Glutamate and GABA content values in the individual 3-min perfusate fractions were calculated by dividing the amount of respective neurotransmitter in the perfusate by the amount of protein in the respective hippocampal slice. Basal neurotransmitter efflux was calculated by averaging the values for the two pre-stimulus basal fractions. Stimulated neurotransmitter efflux was calculated as the sum of total efflux minus basal efflux for each of the three fractions collected for that stimulus, and expressing the data as the fold-increase above basal efflux.

Statistical Analyses

The data are presented as group means±S.E.M. Pregnancy-outcome parameters were analyzed by one-way analysis of variance (ANOVA) coupled with Newman-Keuls test for multiple comparisons. There was no evidence of sex differences, and sex did not interact with treatment on any behavioral measure. Thus, the data for male and female subjects in a treatment group were combined for all statistical analyses. Morris water maze data were analyzed by repeated-measures two-way ANOVA with day as the within-subject factor. For the Conditioned Responding experiments, the number of days to reach criterion for the CRF schedule was analyzed using a one-way ANOVA with treatment as the between-subjects factor. Extinction data were analyzed in a three-way repeated measures ANOVA with treatment group (ethanol, sucrose, water) as the between-subjects variable, and day of training (one to four) and lever (reinforced, non-reinforced) as within-subjects variables. When the assumption of sphericity was violated, a Huynh-Feldt correction was used to adjust the degrees of freedom. All statistically significant effects and interactions of interest were analyzed further using an estimated marginal means procedure with a Bonferroni correction for multiple comparisons. Stimulated neurotransmitter efflux data were analyzed by two-way ANOVA for effects of treatment and stimulus (intensity, or temporal relationship to tetanus), followed by two-tailed, unpaired t-tests

(95% confidence interval) at each stimulus value. P value less than 0.05 was considered to be statistically significant.

Results

Pregnancy Outcome

Pregnancy outcome data are shown in Table 2. CPEE, via chronic oral administration of 3 g ethanol/kg maternal body weight/day, had no effect on average litter size or distribution of male and female offspring (Table 2). Length of gestation increased in the ethanol treatment group compared with the isocaloric-sucrose/pair-fed and water treatment groups. There was one instance of maternal death in the ethanol treatment group at parturition. Perinatal death occurred in one isocaloric sucrose/pair-fed litter, in which two of the four offspring died at birth. There was one instance of spontaneous abortion in each of the ethanol and water treatment groups, and two instances in the isocaloric-sucrose/pair-fed group. Maternal blood ethanol concentration, determined on GD 57 at 1 h after the second divided dose, was 244±11 mg/dl (n=7, range 208-300 mg/dl).

Morris Water Maze

The chronic maternal ethanol regimen did not affect task acquisition in the Morris water maze (Fig. 1). Probe trials administered seven days after completion of the training (acquisition) phase did not reveal differences among the offspring of the three treatment groups (Fig. 1).

Conditioned Responding

Continuous Reinforcement (CRF) Training

The mean number of days to reach criterion for offspring in the ethanol, sucrose, and water treatment groups was 9.88 $\pm$1.81, 11.17 $\pm$3.59, and 10.17 $\pm$ 1.96, respectively. There was no difference in this measure among the three groups ($F(2, 20) = 0.07$, $p > 0.05$).

Extinction

Figure 2 shows the number of reinforced and non-reinforced lever presses across four days of extinction for animals in each of the three treatment groups. The ANOVA revealed a

day-by-lever-by-group interaction ($F(5, 47) = 3.06$, $p < 0.05$). Lever responding decreased over the four days of training in all groups ($F(3, 51) = 8.39$, $p < 0.05$); however, the effect interacted with lever ($F(2, 47) = 9.38$, $p < 0.05$). Post hoc tests showed that this lever-by-day interaction was due to the fact that non-reinforced lever presses did not change across days, whereas reinforced lever presses were higher on days one and two than on days three and four ($p < 0.05$).

All offspring responded more on the previously reinforced lever than on the non-reinforced lever ($F(1, 20) = 35.78$, $p < 0.05$), an effect that interacted with treatment ($F(2, 20) = 4.61$, $p < 0.05$). Post hoc tests for this lever-by-treatment interaction revealed that the offspring of the ethanol group had a higher number of presses on the previously reinforced lever compared with the water and sucrose groups ($p < 0.05$). The offspring of the water and sucrose groups did not differ in the number of previously reinforced lever presses, and there were no group differences in responding on the non-reinforced lever.

During extinction sessions, responses of animals in the three treatment groups were different ($F(2, 20) = 4.04$, $p < 0.05$), and this effect interacted with day ($F(5, 51) = 3.74$, $p < 0.05$). Post hoc analyses revealed that, on the first day of extinction, offspring of the ethanol group responded more than the animals of both the sucrose and water groups ($p < 0.05$), whereas there was no difference in responding for offspring in the water group and sucrose group. On the second day of testing, animals in the ethanol group responded more than those in the water group ($p < 0.05$). There was no difference in the rate of responding between offspring in the sucrose and water groups, or between offspring in the sucrose and ethanol groups. Responses of animals in the three treatment groups for the third and fourth days of extinction were not different.

Amino Acid Neurotransmitter Efflux in Hippocampal Slices

The basal efflux of glutamate and GABA was not different among the treatment groups (Table 3). The data for the isocaloric-sucrose/pair-fed and water treatment groups were not different for any of the parameters measured; hence, these control treatment groups were collapsed into a single control group for the purpose of data analysis. The relationship between the strength of the electrical stimulus and the efflux of glutamate and GABA (input-output relationship) is shown in Figure 3. Neither the glutamate input-output relationship nor the GABA input-output relationship was affected by CPEE. High frequency, stimulus-induced potentiation of glutamate efflux in hippocampal slices (Fig. 4A) obtained from CPEE offspring was not different from that in control hippocampal slices ($F(1,52) = 2.76$, $p = 0.103$). However, high frequency stimulus-induced potentiation of GABA efflux (Fig. 4B) was higher in hippocampal slices obtained from CPEE offspring compared with control animals ($F(1,48) = 9.74$, $p = 0.003$). This difference was most evident at the 5-min time point, although evoked GABA efflux was still elevated at 15 min after high frequency stimulation of the tissue.

Discussion

The guinea pig was chosen as the experimental animal model of human prenatal ethanol exposure because of its extensive prenatal brain development (Dobbing and Sands, 1979). The timing of brain development is species-dependent, and therefore, putative critical periods of vulnerability to ethanol exposure probably vary across different animal species. The most rapid period of brain growth and development, the brain growth spurt (BGS), is one such putative critical period of vulnerability to ethanol. The BGS is a perinatal event in the human, a prenatal event in the guinea pig and sheep, and a postnatal event in the rat and mouse (Dobbing and Sands, 1979). The guinea pig, therefore, is an appropriate animal model for human prenatal ethanol exposure, in which brain development may be affected by maternal oral ingestion of ethanol and subsequent transfer of ethanol across the placenta into the fetal compartment. The guinea pig has a placenta that is most similar to that of the human among rodent species, and has a trimester-equivalent type of gestation that is similar to that of the human (Martensson, 1984).

Chronic prenatal ethanol exposure (CPEE), via maternal ethanol ingestion, results in persistent alterations in brain morphology, neurochemistry and behaviour. Offspring exposed to ethanol during gestation demonstrate deficits in spatial tasks sensitive to hippocampal damage, such as the Morris water maze (see review, Berman and Hannigan 2000), suggesting that CPEE perturbs synaptic plasticity in the hippocampus. In our laboratory, previous studies have demonstrated that CPEE can induce deficits in both Morris water maze performance and long-term potentiation (LTP) in the CA1 region of the hippocampus (Richardson et al. 2002, Iqbal, 2003). Coincident pre- and post-synaptic activity is required for the generation of LTP, and recent studies have provided evidence that the increased excitability in hippocampal circuits that characterizes LTP requires changes in both excitatory and inhibitory mechanisms (Lu et al., 2000). Specifically, an N-methyl-D-aspartate (NMDA) receptor-dependent inhibition of $GABA_A$

receptor-mediated inhibitory postsynaptic potentials may be a required step in LTP induction (Lu et al., 2000). Furthermore, enhancement of GABAergic function in the hippocampus can inhibit LTP induction (Levkovitz et al., 1999).

In this study, we report for the first time that CPEE induces alterations in the evoked release of the inhibitory neurotransmitter, GABA, in the hippocampus of postnatal guinea pig offspring. An increase in post-tetanic potentiation of electrically stimulated GABA release could explain, at least in part, the deficits in hippocampal LTP previously observed in CPEE offspring (Richardson et al., 2002). Interestingly, chronic ethanol exposure in the adult rat that is associated with long-lasting deficits in hippocampal LTP, also increases electrically stimulated GABA release in the hippocampus (Tremwell et al., 1994; Peris et al., 1997). This increase in hippocampal GABA release after chronic ethanol exposure has been attributed to a deficit in presynaptic inhibition of GABA release mediated by $GABA_B$ receptor activation (Peris et al., 1997). Thus, one possible explanation for the observations made in this study is that CPEE results in decreased $GABA_B$ receptor-dependent presynaptic inhibition of GABA release from interneurons in the hippocampus of postnatal offspring. This deficit would be most evident during periods of high-frequency stimulation, such as during the induction of LTP, when $GABA_B$ receptor-mediated inhibition of GABA release plays an important role in facilitating NMDA receptor activation. Future studies will be aimed at elucidating the effects of CPEE on the expression and function of $GABA_B$ receptors in the guinea pig hippocampus.

CPEE, for the maternal ethanol regimen employed in this study, did not alter task acquisition in the Morris water maze. Previous studies conducted in our laboratory have demonstrated that a higher daily dose of ethanol administered throughout gestation (4 g ethanol/kg maternal body weight/day) reliably induces deficits in task acquisition in the fixed-platform version of the Morris water (Richardson et al. 2002, Iqbal et al. 2003). Abdollah et al.

(1993) demonstrated that CPEE, at 4g ethanol/kg maternal body weight/day, resulted in greater behavioural dysfunction, manifesting as increased spontaneous locomotor activity, compared with 3 g ethanol/kg maternal body weight/day, suggesting that CPEE-induced deficits in performance in spatial learning tasks may be dose-dependent. Similar dose-dependent effects of CPEE on spatial task performance also have been observed in the rat (Savage et al. 2002). In adult rat offspring exposed to ethanol during prenatal development, at a maternal blood ethanol concentration of 83 mg/dL, performance in the fixed-platform version of the Morris water maze was unaffected, whereas learning in a "moving-platform" version of the test was markedly impaired (Savage et al. 2002). It will be of interest to determine whether the moving-platform version of the Morris water maze reveals performance deficits in guinea pig offspring exposed to the lower chronic maternal ethanol regimen employed in this study. Indeed, one of the goals of the current study was to determine whether a chronic maternal ethanol regimen, which is lower than that employed in previous studies, would induce neurobehavioural deficits in our animal model that would be revealed by more sophisticated operant-learning paradigms.

In contrast to the spatial navigation task, CPEE induced a pronounced behavioural deficit in an operant conditioning paradigm that manifested as high magnitude of responding on the previously reinforced lever during extinction trials. This result is remarkably consistent across multiple learning paradigms that measure adaptive response inhibition in experimental animals (Abel, 1982; Riley et al., 1979,1980; Driscoll et al., 1990) and children with fetal alcohol spectrum disorder (Mattson and Riley, 1998; Nanson and Hiscock, 1990; Streissguth et al., 1984), indicating that common neuroanatomical structures and/or neurophysiological processes may be involved. Our data suggest that CPEE alters the regulation of presynaptic GABA release, resulting in increased release of this neurotransmitter, in the hippocampus during periods of high-frequency stimulation that would be consistent with deficits in the expression of synaptic

plasticity in this brain region. However, other brain structures that also are affected by CPEE may contribute to the behavioural deficits observed in the present study. In particular, a recent study in the rat (Mihalick et al., 2001) showed that CPEE produced neuronal cell loss in the medial prefrontal cortex that was correlated with deficits in a reversal learning paradigm. Such an effect also may account for, or contribute to, the resistance to extinction observed in CPEE guinea pig offspring. It is likely that CPEE-induced injury of multiple brain structures contributes to the learning deficits, alterations in organizational skills, and poor response to changing environments that characterize individuals exposed to ethanol during prenatal life. Moreover, the results of this study support the idea that CPEE regimens, which do not induce gross impairment in motor and spatial learning skills, nevertheless produce learning deficits that are directly dependent on the complexity of the task.

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Footnotes

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b) Reprint requests to: Dr. J.N. Reynolds, Pharmacology & Toxicology, Botterell Hall Room 535, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

c)

[1]Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

[2]Department of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

[3]The Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Figure Legends

Figure 1: Chronic prenatal ethanol exposure at a dose of 3 g ethanol/kg maternal body weight/day throughout gestation did not impair task acquisition in the Morris water maze. Data are mean ± S.E.M for 8 offspring from 7-8 litters in each treatment group.

Figure 2: Chronic prenatal ethanol exposure (E) increased responding during extinction trials compared to both isocaloric sucrose/pair-feeding (S) and water (W) treatments. Individual points represent the mean ± S.E.M. number of lever presses on the previously reinforced lever (+) and non-reinforced lever (-) during four 30 min sessions.

Figure 3: Chronic prenatal ethanol exposure did not change the input-output relationship for electrically stimulated glutamate (A) or GABA (B) release in the hippocampus of the postnatal guinea pig. Data are mean ± S.E.M. for 5-8 animals from 5-8 litters in each treatment group.

Figure 4: Chronic prenatal ethanol exposure did not alter post-tetanic potentiation of stimulated glutamate release in the hippocampus of the postnatal guinea pig (A). In contrast, post-tetanic potentiation of stimulated GABA release was significantly elevated (B). Data are mean ± S.E.M. for 6-8 animals from 5-7 litters in each treatment group. * GABA release was significantly increased in the ethanol treatment group compared to control at the 5 min post-tetanus stimulus time-point (t-test for unpaired data, $p = 0.036$).

Tables and Figures

Table 1: Subjects in each treatment group not reaching criterion at each stage of training

Group	MT	CRF	FR3-FR7	FR9-FR13	FR15-FR19	FR21-FR25	FR27-FR31
Water	1	3	1	1	3	--	--
Sucrose	3	3	--	1	1	--	--
Ethanol	0	3	--	--	1	2	--

Note. Dashed lines indicate that all animals within the group completed the block of schedules.

MT = magazine training, CRF = continuous reinforcement, and FR = fixed ratio.

Table 2: Pregnancy outcome data

Prenancy Outcome Variable	Treatment		
	Ethanol (7)	Sucrose (8)	Water (7)
Maternal Death	1	0	0
Spontaneous Abortion	1	2	1
Perinatal Death	0	1	0
Length of Gestation (days)	68.9±0.46*	66.8±0.30	66.7±0.84
Litter Size	2.7±0.4	4.0±0.1	3.1±0.7
Male Littermates (%)	55±12	41±7	51±12
Female Littermates (%)	45±12	59±7	49±12

The number of pregnant guinea pigs (litters) in each treatment group is reported in parentheses. The data for maternal death, spontaneous abortion and perinatal death are reported as the number of occurrences. All other data are reported as group means ± S.E.M. of average littermate values for individual litters.

* Increased gestation length in the ethanol-treated group compared with each of the isocaloric sucrose/pair-fed and water-treated groups $F(2,19)=4.804$, $P=0.0205$.

Table 3: Basal glutamate and GABA release per mg protein

Neurotransmitter	Basal release (ng/mg protein)		
	Ethanol	Sucrose	Water
Glutamate	17.7±4.5	12.6±2.5	20.2±7.8
GABA	31.0±7.0	26.2± 5.9	30.8±8.3

Data are mean ± S.E.M. for 8 offspring obtained from 7-8 litters in each treatment group.

Figure 1

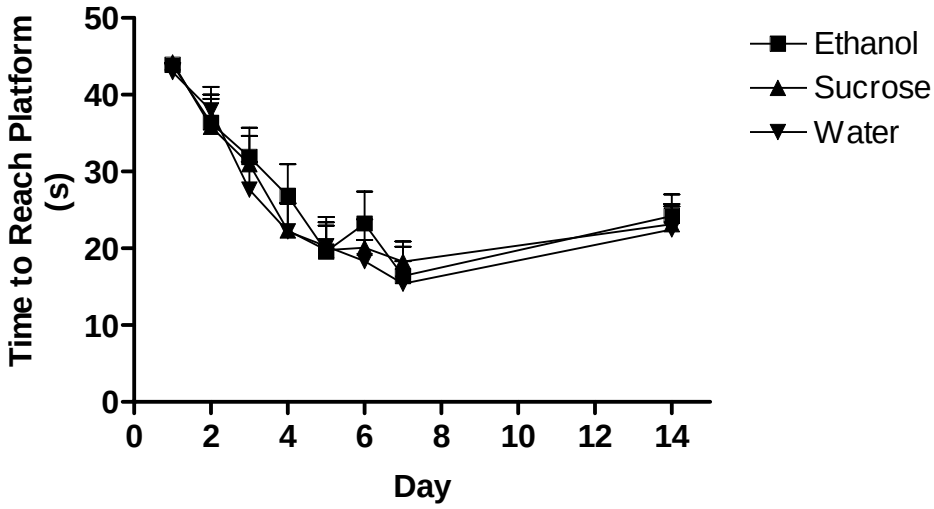

Figure 2

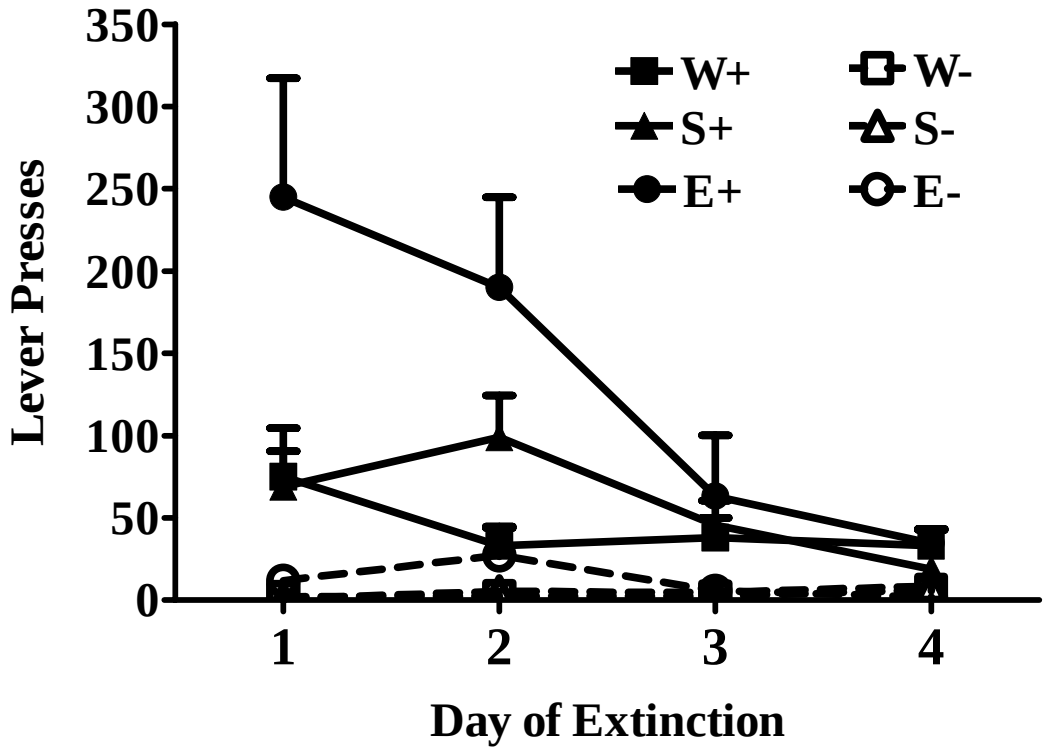

Figure 3

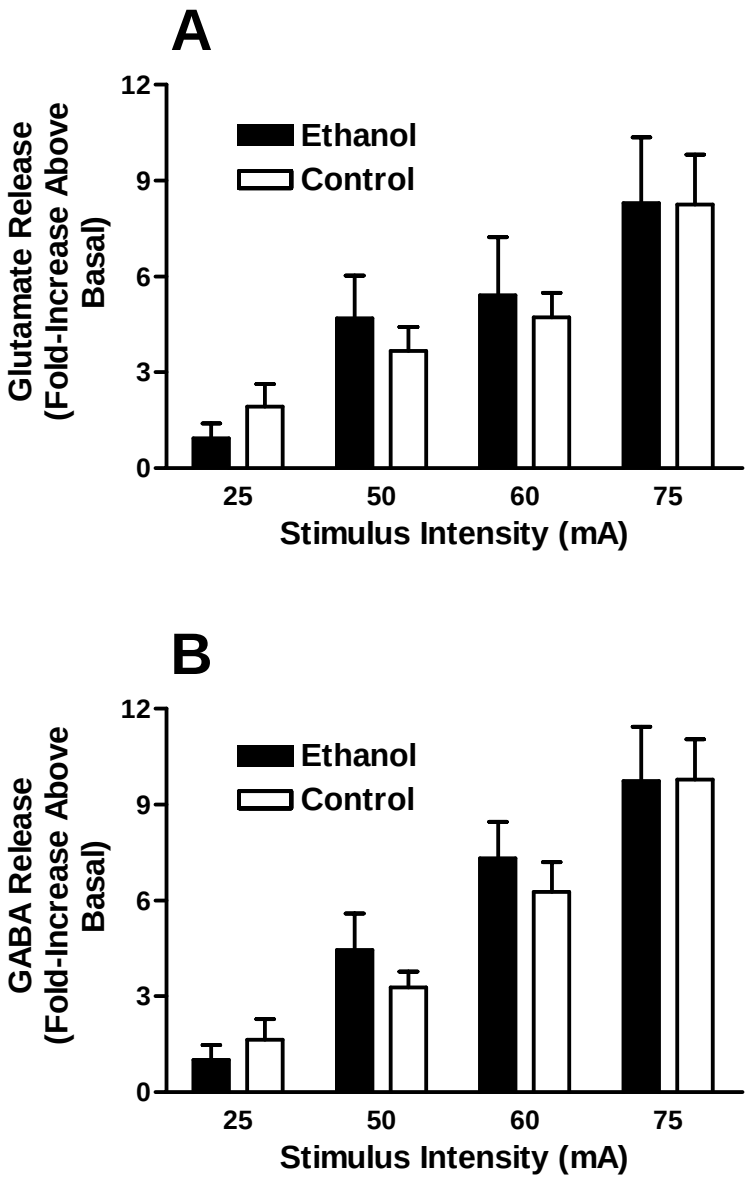

Figure 4