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Prenatal alcohol exposure (PAE) and adolescent stress: Unmasking persistent attentional deficits in rats

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Abstract

Prenatal alcohol exposure (PAE) can produce a myriad of deficits. Unfortunately, affected individuals may also be exposed to the stress of an adverse home environment, contributing to deficits of attentional processes that are the hallmark of optimal executive function. Male offspring of *ad libitum*-fed Control (Con), Pairfed (PF), and PAE dams were randomly assigned to either a five day period of variable chronic mild stress (CMS) or no CMS (Non CMS) in adolescence. In adulthood, rats were trained in a non-match to sample task (T-maze), followed by extensive assessment in the five-choice serial reaction time task (5-CSRTT). Once rats acquired the 5-CSRTT (stable accuracy), rats were tested in three challenge conditions, 1) increased sustained attention, 2) selective attention and, 3) varying doses of *d*-amphetamine, an indirect dopamine and norepinephrine agonist. At birth and throughout the study, PAE offspring showed reduced body weight. Moreover, although PAE were comparable to Con animals in task acquisition, they were progressively less proficient with transitions to shorter stimulus durations (decreased accuracy and increased omissions). Five days of adolescent CMS increased basal corticosterone levels in adolescence and disrupted cognitive performance in adulthood. Further, CMS augmented PAE-related disturbances in acquisition and, to a lesser extent, disrupted attentional processes in Con and PF animals as well. Following task acquisition, challenges unmasked persistent attentional difficulties resulting from both PAE and adolescent CMS. In conclusion, PAE, adolescent CMS, and their interaction produced unique behavioural profiles that suggest vulnerability in select neurobiological processes at different stages of development.

Keywords

5-CSRTT; executive function; cognition; attention; FASD

Introduction

A broad range of behavioural and cognitive impairments are associated with prenatal alcohol exposure (PAE) and fall under the umbrella term Fetal Alcohol Spectrum Disorder

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(FASD) (Mattson et al., 1999). Deficits in executive function (EF) may be attributable to direct and indirect effects of PAE that together produce a myriad of structural and functional alterations (Miller, 1987, Roebuck et al., 1998, Roussotte et al., 2010), potentially altering the trajectory of brain development, as well as increasing vulnerability to further environmental insult. Indeed, postnatal influences may either ameliorate or augment PAE-related outcomes. In humans, trauma, such as postnatal abuse or neglect, accentuate adverse PAE effects (Henry et al., 2007). Thus, an extension of the window of vulnerability can extend beyond the prenatal period, increasing the influence of postnatal adversity.

Adolescence is also a vulnerable period of brain development, with adolescent adversity negatively influencing PAE outcomes. As an example, PAE in humans (Haley et al., 2006, Jacobson et al., 1999) and animal models (Weinberg and Gallo, 1982, Hellemans et al., 2010a) is linked to dysregulation of the fetal stress response system. Programming of the stress system can also occur in adolescence, however (Wright et al., 2008), and pre-existing stress system dysregulation potentially accentuates the impact of adolescent adversity. The long-term effects of adolescent stress on cognitive development into adulthood is relatively understudied, however (McCormick et al., 2008).

With this said, some cognitive processes appear especially sensitive to PAE; more so than would be expected based on IQ scores alone (Connor et al., 2000). For instance, deficits in attention and working memory processes are consistently associated with PAE (Streissguth et al., 1991, Mattson et al., 1999, Green et al., 2009). Indeed, unique attention deficits related to FASD are dissociable from deficits of comorbid disorders like ADHD (Coles et al., 1997, Mattson et al., 2013). Individuals with FASD also prominently display impulsivity that adds to some behavioural impairment, especially those involving selective attentional processes. However, impulsivity is not a unitary process and involves a range of behaviours including the inability to withhold a response and intolerance to delays in gratification/reward (Winstanley et al., 2004). Of interest, the neurotransmitters dopamine, as well as norepinephrine, plays a crucial role in the regulation of these attentional processes. Moreover, PAE-related dopamine and norepinephrine dysfunction is well-noted (Shen and Chiodo, 1993, Shen et al., 1999, Druse, 1981) and reversible in part by stimulants (Wang et al., 2006, Choong and Shen, 2004), suggesting a mechanism by which PAE may alter executive processes like attention.

Here we investigated potentially distinct PAE and adolescent stress-related alterations in attentional processes and impulsivity using the five-choice serial reaction time task (5-CSRTT), a task used extensively to characterize aspects of executive function including attention and inhibitory control (Bari et al., 2008). Task acquisition and performance was assessed during various challenges that taxed sustained (prolonged inter-trial interval) and selective (intermittent tone distracter) attention, and during an indirect behavioural assay of dopamine function with varying doses of *d*-amphetamine.

Materials and methods

Animals

All procedures for the care and use of animals were approved by the University of British Columbia Animal Care Committee, and conducted in accordance with the guidelines set out by the Canadian Council for Animal Care (CCAC), and the US National Institutes of Health. Adult male and female rats were obtained from Harlan Laboratories (Kent, WA, USA), and maintained in the breeding facility at the Center for Disease Modeling at the University of British Columbia, Canada. On the first day of pregnancy (confirmed by the presence of sperm), designated as gestational day (G) 1, dams were separated from the male, singly housed, provided with standard rat chow, and transported from the breeding colony to the investigator colony suites. At this time, dams were randomly assigned to one of three prenatal treatment groups: Control (Con), Prenatal alcohol exposed (PAE), or Pairfed (PF). The Con dams had *ad libitum* access to water and rat breeding chow (19% protein content) throughout gestation, whereas the PAE dams had *ad libitum* access to water and a liquid diet (prepared by Dyets Inc. Bethlehem, PA, Weinberg/Keiver High Protein Experimental Diet #710324) of which 36% of calories were derived from 95% ethanol. In the PF group, each dam was yoked to a PAE dam and provided control liquid diet (maltose dextrin isocalorically substituted for ethanol) in the same amount consumed by their PAE partner (g/kg/body weight/gestation day). Consumption was recorded daily and fresh diet presented approximately 1 hour before lights off each day to reduce disturbance of corticosterone circadian rhythms, particularly in the PF dams that consumed a restricted ration of diet (Krieger; Gallo and Weinberg). Dams remained on the experimental diets until G21 at which time PAE and PF dams were placed on the 19% protein breeding chow consumed by Con dams. Throughout gestation and lactation, dams were single housed in standard, Allentown plastic rat cages with environment controlled filtered lids and maintained in a controlled environment (21°C) on a 12/12 light/dark cycle (lights on 0800 - 2000 h). On the day of birth, postnatal day (PND) 1, pups were weighed and litters culled to about ten (five females, five males when possible). Each week thereafter until weaning (PND 8, 15 and 22), cages were changed and dam and pup weights recorded (average weight/sex/litter for pups). On PND 22, pups were weaned, weighed, ear notched, and housed with same-sex littermates. On PND 35, the pups were pair-housed with a same-sex non-littermate partner from the same prenatal group. At this time, the male pups used in the current study (when possible two male pups from each dam) were randomly assigned to either a CMS (chronic mild stress) or Non CMS (non-stressed) condition.

Adolescent CMS and corticosterone (CORT) sampling

The current study used a modified CMS paradigm developed by the Weinberg laboratory (Hellems et al., 2010b) that entailed exposure to an unpredictable stressor (varying time of day and stressor) twice daily for five consecutive days. CMS began in adolescence at about PND 38. The observance of preputial separation, a marker of the onset of puberty that precedes the onset of high circulating levels of testosterone in male rats (Korenbroet et al., 1977), was used as affirmation that the subjects were adolescents during the CMS regimen.

Blood samples from the tail vein were first collected from all animals under basal (non-activated) conditions on the morning of PND 38. This marked the beginning of the 5-day CMS regimen and subjects assigned to the CMS condition housed separately in a stress-colony room at this time. On the morning following CMS, a second basal blood sample was obtained from all animals in both housing conditions.

The CMS stressors consisted of; social isolation (pups were single housed in mouse cages for the duration of the dark cycle), elevated platform (pups were placed/left on 1 metre high platforms for 10 min in a brightly lit room), 2 h in a tilted cage (30% incline), 30 min of restraint stress (pups were restrained in PVC tubes with holes in the end-caps for air), 1 h in a “novel” cage (a cage without bedding, food or water), 1 h in a “soiled” cage (a home cage of unfamiliar rats) and, a 2 min period of tail-pinching (a piece of flexible rubber tubing was placed over the base of the tail and a plastic clothes pin was then attached). Stress exposure occurred in a room separate from the colony room(s). Animals returned to the main colony room at the end of the CMS period and left undisturbed for about two weeks before testing commenced.

Corticosterone radioimmunoassay

Levels of total plasma CORT, bound and free, were measured using a commercial RIA kit from MP Biomedicals, (Solon, OH) with [125 I] corticosterone as the tracer. The cross-reaction of the antiserum in this kit is 100% for corticosterone. Minimum detectable corticosterone concentration was 0.63 μ g/dl and the intra- and inter-assay coefficients of variation were 1.55 % and 4.26 % respectively.

Cognitive tasks

T-maze: Animals were tested on a version of the non-match to sample T-maze task prior to being assessed on the 5-CSRT, in part, as a means to provide a general index of baseline cognitive performance. The T-maze apparatus consisted of an entry alley (52 L $\times$ 20 W $\times$ 30 H cm), with two arms (50 L $\times$ 20 W $\times$ 30 H cm) that extended in opposite directions at the end of the entry alley. For a more detailed task description see Deacon and Rawlins (Deacon and Rawlins, 2006). In short, animals were group-habituated in a T-maze apparatus for 10 min per day, progressing to individual habituation. By the tenth day, most animals manoeuvred well in the maze and readily consumed pieces of Kellogg's Froot LoopsTM placed at the end of each arm of the maze. During this individual habituation period, the rats were handled multiple times by the experimenter thereby mimicking the pre-test and test phase conditions that occurred during the actual testing period. Once habituated, each animal went through a maximum of nine consecutive days of testing with ten trial blocks per day. Each trial block consisted of a “sample” or “forced choice” in which only one arm of the maze was made accessible, followed 30 s later by a “test” or “free choice” component in which the previous block was removed and the animal was required to choose the correct arm (the arm opposite that in the forced choice condition). The total errors made during the session were recorded daily. The criterion for the T-maze was set at four consecutive days of an averaged 80% correct response.

5-CSRTT: To conduct this task, pair-housed animals were transported by animal care personnel from the original colony at CDM to a colony maintained by the Psychology department at the Kenny building and placed on a reverse 12 h light-dark cycle (lights off at 0800 h) in a climate controlled (21°C) colony room. Animals initially were provided *ad libitum* access to laboratory rat chow and water, and left to acclimate to the new environment for ten days with minimal disturbance. The week prior to the start of training, animals were food-restricted and maintained at about 85 –90% of free feeding weight (about 14 g of rat chow/day per animal) with *ad libitum* access to water. Animals habituated to the 5-CSRTT chambers for 15 min/day for two days and allowed to consume sugar pellets placed in the response wells and food magazine. Full details of the task and protocols can be accessed elsewhere (Carli et al., 1983, Winstanley et al., 2010). Briefly, the task utilized sixteen 5-hole operant chambers (Med Associates, St. Albans, VT, USA) utilizing software written in Med PC by CAW. Rats were trained to nose-poke into a response well upon short illumination of a light located within the target well. The location of the target varied amongst the five wells randomly across trials. Daily sessions consisted of 100 trials with each initiated by the rat responding with a nose-poke into the food magazine. Once initiated, there was a short intertrial interval (ITI) of 5 s, followed by the illumination of one of the five lights. After the light (stimulus) was presented, a limited time (limited hold period) was available in which a response into the well ('correct' response) resulted in the illumination of the food magazine at the back of the chamber and a delivery of a reward (sugar pellet). The light remained on until the animal collected the reward. Responses made prior to the illumination of the stimulus were recorded as 'premature' (impulsive) and punished by a 5 s timeout in which the house light was illuminated. Similarly, an 'incorrect' or lack of response ('omission') resulted in no reward and the initiation of a timeout. Perseverative responding at the correct well was recorded but not punished. Stimulus duration (illumination of the light in response wells) was set at 30 s at the start of training and progressively reduced (20 s, 10 s, 5 s, 2.5 s, 1.25 s) to 1 s, which was the final stimulus duration utilized in the current study. Upon reaching criterion at any particular stimulus duration, the animal graduated to the next duration phase on the following day. For durations 30 s and 20 s, the limited hold was 30 s with a 2 s ITI that also reduced progressively to a 5 s limited hold and 5 s ITI. Criterion for task acquisition was met once individual animals reached the 1 s duration phase. Task acquisition with increasing task difficulty was assessed by comparison of the last session of each duration phase with the first session of the subsequent duration phase. As animals reached the 1 s phase, they were maintained at this stage until all animals had met task acquisition criterion.

Acute Challenges

The 5-CSRTT provides a wide range of measures related to attentional processes involved in EF (Bushnell and Strupp, 2009a). Two acute challenges were incorporated to investigate specifically sustained and selective attention. These additional paradigms, the first, an extension of the ITI period from 5 s to 7 s, and the second a tone distracter, were incorporated immediately following task acquisition.

ITI Challenge

A variation in the timing of the visual cue (the pre-cue delay or Intertrial interval) from 5 s to 7 s for the challenge required the animal to withhold making a response for an additional 2 s before the illumination of the stimulus light. This paradigm allowed for an independent assessment of sustained attention (the ability to remain vigilant), and impulsivity (the inability to withhold a response) (Bushnell and Strupp, 2009b).

Tone Challenge

A second baseline session was added following the ITI challenge to separate the two challenges. An auditory tone was then presented randomly on intermittent (12 times) trials during the interval between trial onset and cue presentation. This allowed for a measure of the engagement or selective attention of subjects during distraction.

Amphetamine

In the current study, we utilized low doses of *d*-amphetamine mean to challenge aspects of attention or impulsivity in well-trained animals without producing noticeable differences in accuracy (Robbins, 2002, Cole and Robbins, 1987). Utilizing a balanced Latin square design (A-D), animals received intraperitoneal (i.p.) injections of vehicle (A=0.9% sterile saline) or varying doses of *d*-Amphetamine hemisulfate (B=0.3 mg/kg, Con=0.6 mg/kg or D=1.0 mg/kg) in a volume of 1ml/kg vehicle solution purchased from Sigma-Aldrich (Oakville, ON, Canada). The doses were calculated as the salt, prepared fresh on the day of injection, and given intraperitoneally 10 min prior to the start of behaviour testing as reported previously (Zeeb et al., 2009, Zeeb et al., 2013). Injections occurred on a three-day schedule that started with a baseline day, followed by a “drug” (saline or amphetamine) day, and then a “day off” prior to the next three-day schedule. This schedule repeated until all rats received each of the possible doses once.

Statistical analysis

All analyses were performed using PASW Statistics for Windows (version 18.0. Chicago: SPSS Inc.). ANOVAs were used throughout and pairwise comparisons conducted when *F* ratios were significant. To be more conservative, the Sidak correction was incorporated for all ANOVAs to adjust for multiple comparisons. Homogeneity of variance was assessed for each test using the Mauchly sphericity test and when violated, the more conservative Huynh-Feldt correction was used to calculate *P*-values for the *F* ratios. In the latter case, *dfs* were rounded for ease of presentation. Significance was set at $P = 0.05$, marginal significance at $P = 0.079$, and trends at $P = 0.110$.

Performances for each PAE group (Non CMS and CMS) were compared with the appropriate control and PF groups. Briefly, we analyzed the percentage of correct and omitted trials, as well as premature responses. In addition, analyses were performed on sum perseverations, completed trials, and latency to respond and collect for correct responses. Analysis of task acquisition entailed a comparison of the last session of particular stimulus duration (previous) with the initial (first) session of the subsequent stimulus duration and referred to as “transition”.

Results

Maternal weights

Although all dam weights were comparable at the start of the prenatal treatment, both PAE and PF dams had lower absolute body weights relative to Con dams by the end of gestation. Once corrected for percent gain from the start of gestation, however, only dams in the PAE group had a significantly reduced percent of weight gain (Table 1).

One-way ANOVAs showed no significant effect of Treatment on dam weight at G1 [$F_{2,22} = .87, P = 0.43$], but a significant Treatment effect at G21 [$F_{2,21} = 4.15, P = 0.03$], and a marginal effect of percent gain [$F_{2,22} = 2.87, P = 0.079$]. Pairwise comparisons with LSD correction for multiple comparisons revealed that whereas PF dams showed only a trend toward reduced gain ($P = 0.108$), PAE dams had a significantly reduced percent gain over the gestation phase ($P = 0.03$).

Offspring weights

At birth, average pup weight was lower in PAE but not PF pups compared to Con. PAE offspring maintained lower weights relative to Con throughout development (Figure 1A) and into adulthood (Figure 1B). PF offspring, on the other hand, had lower body weights than Con in the second week of life only. An ANOVA with Huynh-Feldt correction showed an interaction of Age by Treatment ($F_{3,64} = 6.36, P = 0.001$). Pairwise comparisons revealed that PAE pups had lower weekly weights compared to Con from birth through to weaning (all P s < 0.02). PF animals showed lower weights relative to Con at the end of the second week of life only ($P = 0.012$). ANOVAs revealed a significant main effect of Treatment ($F_{2,47} = 4.44, P = 0.017$), but no main effect of Condition, nor interaction (all P s < 0.60).

Pre- and Post-CMS Corticosterone

The CMS condition produced increases in basal CORT levels in both the Con and PF offspring but not in the offspring of the PAE treatment (Figure 1C). An ANOVA with Treatment and Condition as variables showed a within-subject effect of CORT ($F_{1,47} = 7.40, P = 0.009$), as well as an interaction of CORT and Condition ($F_{1,47} = 7.53, P = 0.009$) and a significant between-subject effect of Condition ($F_{1,47} = 9.62, P = 0.003$). Pairwise comparisons revealed a significant increase from pre- and post- CORT sampling in the Con and PF offspring in the CMS condition only ($P = 0.055$ and $P = 0.001$), as well as showing that Con- and PF- CMS animals had higher CORT levels following CMS than their Non-CMS counterparts ($P = 0.09$ and $P = 0.022$).

T-maze

It is important to note that five animals were unable to meet the requirements needed to acquire the basic elements of the T-maze task and were therefore eliminated from further testing. The eliminated animals included one Control [CMS condition] and four PAE [one Non CMS and three CMS condition]. Using the cross-tabulation output of count and percentage of animals in each treatment to acquire the task, A Pearson's Chi-square showed a trend towards significant impact of Treatment on task acquisition $X^2 (2, N = 53) = 4.99, P = .08$.

Of the animals that trained in the T-maze, Con- Non CMS animals alone showed significant improvement over the course of the 9 days relative to the PF and PAE rats (Figure 2A). Although all groups were equal at the start of testing, the Con- Non CMS group had fewer errors relative to all other groups on the final day in the T-maze.

A repeated measures ANOVA showed a significant main effect of Day ($F_{8,336} = 7.672$, $P < 0.001$), Day by Condition interaction ($F_{8,336} = 2.737$, $P = 0.006$), and a marginally significant, three-way interaction ($F_{16,336} = 1.624$, $P = 0.061$). Pairwise comparisons indicated that Con, but not PF nor PAE, rats in the Non CMS condition had significantly fewer errors on the final day of testing relative to their CMS counterparts ($P = 0.05$, 0.65 , and 0.17 , respectively).

5-CSRTT

Acquisition—Analyses of task acquisition was performed with a repeated measures ANOVA for each measure of interest using Stimulus Duration (Duration), Treatment, Condition, and Transition as variables. Two animals were removed from acquisition analysis; one animal displayed reduced performance (less than 60 trials per session) for an extended period during task acquisition, and a second failed to reach the 1.0 s stimulus duration criterion by the end of the acquisition phase.

Session counts—To assess the pace of acquisition, we recorded the number of sessions required to reach criterion for each of the increasingly shorter stimulus durations to, but not including, the final 1 s duration phase. In summary, PAE- CMS animals displayed a significant delay in reaching criterion in the task (Figure 2B), requiring a greater number of sessions relative to their same treatment counterparts, PAE- Non CMS, and Con rats.

A repeated measures ANOVA showed a marginally significant within-subject interaction of Duration by Treatment by Condition ($F_{4,32} = 2.34$, $P = 0.069$), as well as between-subject interaction of Treatment and Condition ($F_{2,39} = 2.80$, $P = 0.073$). The pairwise comparison revealed no group differences in the Non CMS condition. In contrast, PAE- CMS animals showed a trend towards requiring significantly more trials to criterion at 30 s ($P = 0.10$) relative to Con- CMS, and at 1.25 s relative to Con- and PF- CMS rats ($P = 0.042$; $P = 0.023$, respectively).

Accuracy—PAE animals in both the Non CMS and CMS conditions decreased their accuracy during transitions. Con animals also showed decreased accuracy as well, but only at shorter durations, as was the case, but to a lesser extent, for PF animals. Furthermore, Con and PF animals showed *increases* in accuracy during some transitions, an outcome not observed during any transitions in PAE animals (Figure 3A, B). Overall, CMS *decreased* accuracy during transitions.

A repeated measures ANOVA with Huynh-Feldt adjustment showed a significant interaction of Duration by Transition ($F_{5,364} = 12.75$, $P < 0.001$), and a significant main effect of Transition ($F_{5,364} = 29.50$, $P < 0.001$). There was also a significant between-subject effect of Condition ($F_{2,80} = 4.01$, $P = 0.049$), resulting from a general CMS- related decrease in accuracy. Pairwise comparisons indicated that optimal accuracy occurred across

treatments by the 10 s transition [with 2 second ITI] in the Non CMS condition, and, with the exception of the initial 20 s duration phase ($P < 0.05$); transition accuracy did not differ across treatments (all P 's > 0.1). In contrast, PAE- CMS rats dropped in accuracy at the 1.25 s duration phase ($P < 0.05$). A significant CMS-induced lag in accuracy was also shown at the 20 s duration, with PAE- CMS animals showing reduced accuracy relative to their Con- CMS counterparts ($P = 0.03$). Reduced accuracy occurred across treatment groups, however, especially with the shorter duration periods (Figure 3A and B).

Perseverations—PAE produced increases in perseverations relative to Con- Non CMS and PAE- CMS animals. However, PAE- CMS animals also showed increased perseverations but only at the shortest stimulus duration transition. Neither CMS alone, nor PF (both Non CMS and CMS conditions) resulted in any significant changes across transitions (Figure 3C and D).

A repeated measures ANOVA with Huynh-Feldt adjustment showed a significant interaction of Transition by Condition [$F_{2, 157} = 4.07, P = 0.02$], a marginally significant interaction of Transition by Treatment by Condition [$F_{2, 157} = 2.32, P = 0.06$], as well as a main effect of Transition [$F_{2, 157} = 3.53, P = 0.03$]. Pairwise comparisons revealed that PAE- Non CMS animals showed a significantly greater frequency in perseverations in transition to the 5 s stimulus duration relative to both Con- Non CMS and PF- Non CMS animals ($P = 0.005$; $P = 0.015$, respectively), and at the completion of the 5 s phase ($P = 0.026$) as well relative to Con- Non CMS animals. PAE- CMS rats, on the other hand, showed a significant increase in the percentage of perseverations by the end of the 1.25 s duration phase relative to both Con- and PF- CMS animals ($P = 0.017$; $P = 0.046$, respectively). In addition, PAE- CMS rats showed decreased percentage of perseverations at 10 s, 5 s, and 2.5 s transitions relative to their Non CMS counterparts ($P = 0.08$; $P = 0.02$; $P = 0.015$, respectively), but a significant increase - going from about 1% to 12% perseverations - at the 1 s transition (0.014).

Omissions—Rats in both the Con- Non CMS and CMS conditions had increased omissions during the switch from 2 s ITI to 5 s ITI (5 s Transition), with the Con- CMS group showing an increase in omissions in the 20 s transition as well. In contrast, the PF- Non CMS and CMS animals showed little change in the number of omissions during any of the transitions (Figure 3E, F). On the other hand, PAE- CMS, and to a lesser extent PAE- Non CMS animals, showed a profound change in omissions during transitions, with increased omissions at three out of the six transition points.

Repeated measures ANOVA across the six transition periods showed an interaction of Duration and Transition [$F_{3, 250} = 8.95, P < 0.001$], as well as a main effect of Duration [$F_{3, 250} = 27.07, P < 0.001$], and a between-subject main effect of Transition [$F_{1, 81} = 7.30, P = 0.008$]. Pairwise comparisons revealed that while PAE- CMS showed a trend toward reduced omissions in the first transition ($P = 0.11$), they had significantly more omissions in the first session of subsequent 10 s, 2.5 s, and 1.0 s transitions [$P = 0.005$; $P = 0.006$; $P = 0.063$, respectively].

Premature Responses—Overall, premature responses increased with the decrease in stimulus duration and were amplified by CMS, with all groups showing increases in percent of premature responses (Figure 4A, B).

A repeated measures ANOVA showed a within-subject effect of Duration [$F_{4, 319} = 7.37, P < 0.001$], and a between subject main effect of Condition [$F_{1, 81} = 5.86, P = 0.018$], but no other main effects or interactions.

Correct Latency—Overall, the transition to the shorter stimulus duration phases produced a faster mean response time between the stimulus presentation and a subsequent nose-poke in the correct well (Figure 4C, D). However, PAE and PF animals in the Non CMS condition were significantly faster than Con- Non CMS animals in their response during most stimulus duration phases.

A repeated measures ANOVA showed an interaction of Transition X Duration [$F_{2, 194} = 6.43, P = 0.001$], and a main effect of Duration [$F_{2, 194} = 36.43, P < 0.001$]. As well, there was a significant between-subject main effect of Transition [$F_{1, 80} = 144.38, P < 0.001$]. Pairwise comparisons showed that PAE- Non CMS were faster in responding than Con- Non CMS animals in the final session of 10 s, 5 s, 1.25 s and 1 s duration phases ($P = 0.14, P = 0.01, P = 0.05, P = 0.06$, respectively). A similar effect was revealed in the PF- Non CMS group in the 10 s, 5 s, 2.5 s, 1.25 s and 1 s duration phases ($P = 0.09, P = 0.001, P = 0.07, P = 0.09, P = 0.05$, respectively). However, Con- CMS animals alone showed a significant increased latency relative to their Non CMS counterparts (Figure 4C and D) during the final session of all but the 20 s and 1.25 s duration phases ($P \leq 0.120$).

Collection Latency—In general, transitions to shorter stimulus durations produced a faster mean response time between a nose-poke in the correct hole and collection of the reward (Figure 4E, F). Other than the transition to the 20 s duration phase, this was more pronounced for animals in the Non CMS compared to the CMS condition.

A repeated measures ANOVA showed a within -subject interaction of Transition by Duration [$F_{3, 248} = 5.56, P = 0.001$], and a main effect of Duration [$F_{3, 248} = 23.02, P < 0.001$]. As well, there was a marginally significant between-subject interaction of Treatment by Condition [$F_{2, 80} = 2.96, P = 0.058$], and significant main effects of Treatment [$F_{2, 80} = 3.72, P = 0.029$], and Transition [$F_{1, 80} = 5.00, P = 0.028$].

Tray- Pokes—Tray-pokes did not vary across transitions, although they did show an overall increase with task difficulty across the groups. A repeated measures ANOVA showed only a main effect of tray-poke over Duration [$F_{3, 259} = 10.61, P < 0.001$] with no other main effects (all P s > 0.15) or interactions (all P s > 0.15).

Stable performance—To determine whether animals had successfully acquired the task and reached stable baseline performance, data from the final five sessions of the training phase (sessions 40 - 44) were analyzed with a repeated measures ANOVA of percent correct using two between-subjects factors, Treatment (Con, PF, PAE) and Condition (Non CMS, CMS), and Session as the within - subject factor. As the effect of Session was not significant

in any treatment or condition, all animals were judged to have reached stable performance levels in the task.

To summarize, PAE- CMS animals took longer to reach criterion during the shorter stimulus durations. PAE-CMS also showed an increased sensitivity to decreasing stimulus duration, displaying decreased accuracy relative to both Con and PF - CMS animals and increased perseveration and omissions during the transition to shorter stimulus duration. However, PAE - Non CMS animals did show increased perseverations during the transition from a 2s to 5s ITI relative to Con and PF rats, indicating a PAE-related deficit in inhibitory control. Overall, we can conclude that PAE impairs learning (longer to reach criterion and decreased accuracy) by reducing flexibility (perseverations) and sustained attention (omissions). In contrast, alone, CMS appears to produce deficits in inhibitory control (increased premature responses) and attention (omissions).

Acute Challenges—Once all of the animals had reached stable performance (with the exception of two, as previously noted), they were re-assessed during an ITI challenge (ITI changed from 5 s to 7 s), followed by a second baseline session and then the Tone distracter. Repeated measures ANOVA were used to assess performance of sustained (ITI challenge) and select (Tone challenge) attention for these tasks.

Accuracy—All treatment groups showed a significant decrease in accuracy in response to the ITI challenge, irrespective of adolescent CMS condition. In contrast, there was no change in accuracy in response to the Tone challenge (Figure 5A).

A repeated measures ANOVA with Huynh-Feldt showed a within-subject main effect of challenge Day [$F_{2, 76} = 27.60, P < 0.001$], but no other significant effect (all P 's > 0.10).

Perseverations—PAE animals showed *increased* mean perseverations in basal measures during the acute challenge phase relative to Con rats. Nonetheless, all CMS groups as well as the PAE- and PF- Non CMS groups showed a significant or trend towards significant *decrease* in mean perseverations during the ITI challenge (Figure 5B).

A repeated measures ANOVA with Huynh-Feldt adjustment showed a within-subject main effect of challenge on perseveration [$F_{2, 76} = 7.96, P = 0.001$]. Pairwise analysis indicated that the change from 5 s to 7 s ITI produced a decrease in perseverations in PAE- and Con-CMS, and to a lesser extent PF- CMS, rats [$P = 0.02, P = 0.05, P = 0.06$, respectively]. Pairwise comparison revealed that PAE- Non CMS animals had a tendency for higher perseverations during Baseline, ITI, and Tone sessions [$P = 0.06; P = 0.10; P = 0.05$; respectively] relative to Con- Non CMS rats.

Omissions—CMS produced an increase in omissions during the ITI challenge for Con animals, whereas the PF- Non CMS showed a similar increase in response to the Tone challenge (Figure 5C). In contrast to Con and PF animals, neither challenge influenced the percent of omissions in PAE animals.

A repeated measures ANOVA showed a main within-subject effect of challenge [$F_{2, 78} = 5.54, P = 0.005$], but no interactions. Pairwise analysis revealed a significant increase in

omissions in response to the ITI challenge in Con- CMS animals alone ($P = 0.016$; all other P s > 0.20). In contrast PF- Non CMS animals showed a significant increase in omissions in response to Tone ($P = 0.02$; all other P s > 0.18). PAE animals in both conditions showed no changes in omission (all P s > 0.18).

Premature Responses—Extending the ITI produced an overall increase in the percent of premature responses in all groups (Figure 5D). However, only the control animals showed an influence of adolescent CMS. Con- CMS animals displayed the greatest increase in premature responses, both at baseline and in response to the ITI challenge, and to a lesser extent in response to the Tone challenge, relative to their Non CMS counterparts and, in comparison to the PF and PAE groups as well.

A repeated measures ANOVA with Huynh-Feldt adjustment showed a within-subject main effect of Premature response [$F_{2, 56} = 208.7$, $P < 0.001$], and a trend toward an interaction of Treatment by Condition in the between-subject analysis [$F_{2, 38} = 2.36$, $P < 0.109$].

Pairwise comparisons revealed significantly higher rates of premature responding overall in response to the ITI challenge [all P 's < 0.001]. Similar effects were found in relation to the Tone challenge in Con [$P = 0.088$, $P = 0.041$] and PF [$P = 0.008$, $P = 0.013$] rats in both the Non CMS and CMS conditions, but not in PAE- Non CMS and CMS rats [$P = 0.778$, $P = 0.451$, respectively]. Relative to their Non CMS counterparts, Con- CMS rats had higher premature responses at baseline that was also present in the ITI challenge [$P = 0.04$, $P = 0.039$], but differences were not observed during the baseline and Tone sessions [$P = 0.181$, $P = 0.227$]. Con- CMS rats also displayed a trend towards higher premature responses relative to PF [$P = 0.101$, $P = 0.08$, $P = 0.052$, $P = 0.148$] and PAE- [$P = 0.053$, $P = 0.086$, $P = 0.594$, $P = 0.098$] CMS animals, with the exception of the baseline measure in the latter. To a lesser extent the Con- Non CMS animals showed increased premature responses in the Tone baseline and challenge [$P = 0.05$, $P = 0.120$, respectively], while there was no increase in PAE- Non CMS [$P = 0.131$], nor PF Non CMS [$P = 0.114$] rats in the ITI challenge.

Correct Latency—All rats in the Non CMS condition showed a decrease in time to respond to the stimulus during correct trials with the ITI challenge, but only Con - Non CMS animals showed a similar trend in response to the Tone challenge (graph not shown). Only PF animals showed a differential effect of CMS however, but only in response to the ITI challenge.

A repeated measure ANOVA showed a main effect of challenge [$F_{3, 114} = 10.64$, $P < 0.001$], but no interactions (all P s > 0.20). Pairwise comparisons revealed significant, or marginally significant, decreases in latency in Con-, PF- and PAE- Non CMS and PF- CMS animals ($P = 0.046$, $P = 0.016$, $P = 0.077$, $P = 0.005$, respectively) in response to the ITI challenge.

Collection Latency—All groups showed little variation in collection latency (Figure 5E). A repeated measures ANOVA showed a main effect of challenge day [$F_{3, 105} = 3.49$, $P = 0.02$], but no interactions (all P s > 0.30). There was a trend toward a main between-subject effect of Condition [$F_{1, 38} = 2.96$, $P = 0.058$], with pairwise tests revealing a significant

decrease in latency in response to ITI challenge in PAE- Non CMS animals alone ($P = 0.044$, all other P s > 0.14).

Tray- pokes—The ITI challenge and, to a lesser extent, the Tone distracter, produced an increase in tray-poke responses in Non CMS animals. In contrast, while PAE- CMS animals alone showed an effect of the challenges compared to their PF- and Con- CMS counterparts (Figure 5F), only Con- CMS rats showed significant differences relative to their Non CMS counterparts.

A repeated measures ANOVA with Huynh-Feldt adjustment showed a within-subject main effect of Tray-pokes [$F_{2, 57} = 14.06$, $P < 0.001$], and a between-subject interaction of Treatment and Condition [$F_{2, 38} = 3.65$, $P < 0.036$]. Pairwise comparisons revealed a significant decrease in Tray-pokes in the ITI challenge in Con- Non CMS animals [$P = 0.05$]. PAE- CMS animals, on the other hand, showed a significant decrease in tray-pokes in response to both the ITI and Tone challenges [$P = 0.008$, $P = 0.005$, respectively], although Con- Non CMS animals displayed greater responding to both challenges relative to their PF and PAE counterparts [P s > 0.05].

Total Trials—The ITI challenge produced a significant decrease in valid trials for Con and PF animals in both Non CMS and CMS conditions, but did not influence trials for the PAE animals. On the other hand, CMS produced a marginally significant decrease in valid trials in response to the Tone challenge, but only for PAE animals (graph not shown).

A repeated measures ANOVA showed a significant main effect of challenge [$F_{1, 51} = 22.03$, $P < 0.001$], but no interactions [$F_{1, 51} = 22.03$, $P > 0.20$]. Pairwise analyses showed that Con and PF animals in both Non CMS and CMS conditions had a reduced number of valid trials in response to the ITI challenge (all P s > 0.05), but not the Tone challenge (all P s > 0.19). In contrast, only PAE- CMS animals showed a marginally significant decrease in valid trials in response to the Tone challenge alone ($P < 0.089$, all other P s > 0.19).

In summary, adolescent CMS decreased accuracy during transitions across treatments during the ITI challenge relative to their Non-CMS counterparts, as well as increased omissions and premature responses in Con animals alone relative to their Non-CMS counterparts. Although CMS and prenatal treatments produced a decrease in perseveration during transitions in the ITI challenge, both PAE - Non CMS and - CMS rats showed increased perseverations relative to Con animals as well. Although Con- CMS and both PF Non CMS and CMS showed decreases in perseverations and premature responses during the Tone challenge, PAE animals did not display Tone-related influences on behaviour.

Overall, CMS impaired learning (decreased accuracy) when attention processes were challenged (increased omissions) as well as producing positive alterations in PFC circuitry related to flexibility (reduced perseverations) and inhibitory control (premature), albeit in a context specific manner in the latter case of premature responses. PF produced very similar changes as CMS that did not appear exacerbated by the addition of adolescent CMS. PAE, with or without the addition of CMS, appeared to influence similar systems as CMS alone, however PAE had a negative influence on PFC circuitry related to flexibility (increased

perseverations) and the attention deficits appeared restricted to sustained attention (ITI challenge) rather than selective attention (Tone challenge).

Amphetamine—All animals were responding at or near the maximum 100 trials per session prior to commencing the amphetamine (AMPH) challenge. However, a number of the Con- CMS and PAE- CMS animals significantly reduced responding during the 0.6 mg/kg and 1.0 mg/kg AMPH challenges. To maintain the Ns and allow for valid assessments of performance, a criterion of 20 valid trials was set for measures obtained as a percentage of trial responses. Sum data or mean sum data that included tray pokes and perseverations were not reliable under these circumstances and therefore omitted from further analysis.

Accuracy—All animals were comparable in accuracy across AMPH doses. However, only Con- CMS animals showed a steady dose-related decrease (Figure 6A).

An ANOVA of accuracy with Sidak adjustment for multiple comparisons, and Treatment, Condition and Dose as variables, showed a main effect of Dose [$F_{3, 156} = 3.93$, $P = 0.01$] but no interactions (all P s > 0.30). Overall, 1.0 mg/kg AMPH produced the lowest level of accuracy relative to Sal ($P = 0.005$).

Omissions—PAE produced an increase in omissions with the high dose of AMPH. In contrast, CMS produced opposing responses in PAE and Con animals, with PAE- CMS animals showing lower and Con- CMS showing higher omissions than their Non CMS counterparts (Figure 6B). An ANOVA with Treatment, Condition and Dose as variables showed a trend towards a three-way interaction [$F_{6, 156} = 1.84$, $P < 0.096$], and a main effect of Dose [$F_{3, 156} = 15.94$, $P < 0.001$]. Pairwise comparisons revealed a significant increase in omissions at 1.0 mg/kg relative to all other doses (all P s < 0.05) and showed that both Con- and PAE- Non CMS animals differed significantly from their CMS counterparts, but in opposite directions. There were higher omissions in Con- CMS versus- Non CMS ($P = 0.036$), and lower omissions in PAE- CMS versus PAE-Non CMS ($P = 0.009$). Moreover, PAE- Non CMS animals had significantly higher omissions at the 1.0 mg/kg dose relative to both Con- and PF- Non CMS animals ($P = 0.02$, $P = 0.01$, respectively). In contrast PAE- CMS had significantly fewer omissions than Con- CMS animals ($P = 0.016$).

Premature Responses—PAE alone did not significantly alter the number or rate of premature responding in the AMPH challenge (Figure 6C). However, CMS increased premature responses in PAE relative to their Non CMS counterparts and increased premature responses at higher doses in Con animals. An ANOVA with Sidak correction and Treatment, Condition and Dose as variables showed a significant interaction of Dose and Treatment [$F_{6, 156} = 2.13$, $P = 0.05$], and main effects of Treatment [$F_{2, 156} = 7.13$, $P = 0.001$] and Dose [$F_{3, 156} = 2.13$, $P < 0.001$]. Under Non CMS conditions, PAE and Con animals were comparable (P s > 0.25), although PF animals showed reduced premature response relative to both PAE and Con at the 0.3 mg/kg dose ($P = 0.012$ and $P = 0.008$, respectively), and a trend for lower response at 0.6 mg/kg relative to Con ($P = 0.10$). CMS produced increased premature responding at 0.6 and 1.0 mg/kg doses in Con- relative to PAE- Non CMS ($P = 0.110$; $P = 0.002$, respectively) and, to a lesser extent, PF- Non CMS

animals ($P = 0.44$; $P = 0.009$, respectively). Nonetheless, PAE alone showed a trend towards a significant impact of CMS relative to their Non CMS counterparts ($P = 0.096$; all other P s > 0.20).

Correct Latency—The low dose of 0.3 mg/kg AMPH reduced correct latency to respond for all animals in the CMS condition to levels similar to Con- Non CMS animals. However, as AMPH dose increased so did the latency of Con- CMS and PAE- Non CMS and CMS animals. This was not the case of the PF animals (Figure 6D).

An ANOVA showed an interaction of Treatment by Dose [$F_{6, 160} = 2.25$, $P = 0.04$], and a significant main effect of Condition [$F_{1, 160} = 4.86$, $P = 0.03$] and Dose [$F_{3, 160} = 5.42$, $P = 0.001$], as well as a marginally significant main effect of Treatment [$F_{2, 160} = 2.52$, $P = 0.08$]. Pairwise comparisons showed that PAE- Non CMS showed marginally longer latencies relative to the Con- Non CMS animals at 1.0 mg/kg dose ($P = 0.070$), but no differences were present between Con- and PAE- CMS animals at any dose. Con- CMS also had significantly longer latencies at 1.0 mg/kg relative to their Non CMS counterparts ($P = 0.001$). PF- Non CMS animals on the other hand, showed marginally greater latencies at Sal and 0.3 mg/kg AMPH doses relative to Con- Non CMS animals ($P = 0.088$, $P = 0.066$, respectively). In contrast, PF- CMS animals had significantly longer latencies to start (Sal), but shorter latency relative to the Con- CMS at the 1.0 mg/kg AMPH dose ($P = 0.02$, $P = 0.02$, respectively).

Collection Latency—Overall, PAE- Non CMS alone showed increased collection latency at the 1.0 mg/kg AMPH challenge relative to Con- and PF- Non CMS as well as PAE- CMS counterparts (Figure 6E). An ANOVA with Treatment, Condition and Dose as variables showed a main effect of Dose [$F_{2, 160} = 3.49$, $P = 0.046$], but no other main effects or interactions (all P s > 0.20). Pairwise analysis revealed that 1.0 mg/kg AMPH produced significantly longer collection latencies relative to Sal in PAE- Non CMS animals only ($P = 0.003$). At the 1.0 mg/kg dose, PAE- Non CMS animals also showed longer response latencies relative to their CMS counterparts ($P = 0.004$), and in comparison to the Con and PF- Non CMS animals ($P = 0.001$, $P < 0.001$, respectively).

Total and Valid Trials—Between one and two animals in each treatment were removed from assessment owing to an almost complete absence of response (trials < 19) over the 30 min of testing. Of those that did continue to respond, we saw a significant reduction in trials in both PAE- CMS and PF- CMS, though the most profound affect was in the former (Figure 6F).

A repeated measure ANOVA showed a marginally significant interaction of Dose by Treatment [$F_{5, 97} = 2.29$, $P < 0.056$], and main effect of Dose ($F_{2, 97} = 8.78$, $P < 0.001$). Relative to the Sal dose, there was a significant *increase* in initiated trials (all P s < 0.05) at 0.3 mg/kg AMPH in all treatment groups, with the exception of PAE- CMS animals. In addition, PAE- CMS rats alone showed a *decrease* in initiated trials ($P = 0.03$) at the 1.0 mg/kg dose relative to Sal.

A repeated measure ANOVA of valid (attempted) trials showed an interaction of Dose X Treatment [$F_{5, 111} = 2.49$, $P < 0.032$], and a main effect of Dose [$F_{5, 111} = 37.62$, $P < 0.001$]. The interaction was due to a significant reduction in attempted trials by PAE- CMS animals at the Sal and 0.3 mg/kg doses ($P = 0.05$; $P = 0.03$, respectively) relative to Con- CMS, and a marginally significant decrease relative to PF -CMS animals at the Sal, 0.3, and 1.0 mg/kg doses ($P = 0.06$; $P = 0.056$, $P = 0.057$, respectively). PAE- and Con- Non CMS animals showed a marginally significant decrease in valid trials ($P = 0.056$; $P = 0.056$, respectively) relative to PF- Non CMS animals as well.

In summary, amphetamine increased the number of initiated trials across groups. Adolescent CMS reduced accuracy and increased premature responses and omissions in Con animals during the AMPH challenge. Although CMS also increased premature responses in PAE animals, amphetamine also decreased omissions and attempted trials in PAE animals relative to the Con -CMS animals, whereas PAE- Non CMS increased omissions and collection latency relative to both Con Non CMS and PAE counterparts. The PF group, on the other hand, showed amphetamine-related decrease in premature responses as well as an increase in collection latency.

Overall, the effects of CMS were negatively influenced by enhanced DA availability during the 5-CSRTT, showing decreased performance (accuracy), reduced inhibitory control (premature) and attention (omissions). PF, on the other hand, showed no influence of DA availability on accuracy and actually displayed an increase in inhibitory control (decreased premature). Increased DA availability also did not negatively affect accuracy in PAE animals, although they did display decreased attention (increased omissions) and motivation for reward (increased collection latency). Adolescent CMS altered the effects of PAE alone, decreasing potential attention deficits (decreased omission), but also decreased inhibitory control (increased premature).

Discussion

The present study investigated the influence of PAE and CMS, as well as their interaction, on attentional processes utilizing an in-depth analysis of task acquisition in the 5-CSRTT, followed by analysis on three challenges that potentially interfere with, or facilitate EF. In particular, two of the challenges taxed elements of attention, sustained and selective attention specifically, to unmask subtle attentional deficits, whereas the third challenge, the *d*-amphetamine challenge, was used specifically to test aspects of attentional processes and impulsivity. The results highlight the capacity of PAE to produce persistent alterations in behaviour well into adulthood and beyond. Moreover, the results add to the growing body of evidence implicating the impact of environment, in particular stress during the adolescent period, on an already dysregulated system because of PAE. Indeed, the implications of these results are quite sobering as they underscore the widespread impact of a relatively short (5-day) period of adolescent stress on the developing brain and the long-term consequences on cognitive behaviour in both control and PAE animals, with the latter in particular showing both vulnerability and adaptive plasticity.

In summary, there were seven major findings in the current study: 1) Moderate PAE persistently suppressed growth of offspring; 2) Adolescent CMS selectively increased basal CORT levels; 3) Prenatal alcohol exposure, but not pair-feeding, produced attention deficits but was not associated with overall performance in the 5-CSRTT; 4) The effects of adolescent CMS were prenatal treatment-dependent in both the T-maze and the 5-CSRTT; 5) Acute challenges in adulthood ‘unmasked’ deficits of PAE and adolescent CMS; 6) PAE animals showed a unique cognitive profile, allowing us to dissociate characteristics of PAE from those of pair-feeding effects; and 7) Varying *d*-amphetamine doses produced differential effects in the 5-CSRTT depending on pre- and postnatal environment and cognitive process. Each of the main findings is discussed separately.

Moderate alcohol intake during gestation persistently suppressed growth of offspring

PAE resulted in lower body weight of offspring at birth. In contrast to the PF offspring, which showed only a short-term transient decrease in body weight, PAE animals did not show “catch-up” growth, maintaining lower weights throughout the study. Lowered body weights often have been reported in PAE offspring at various times during lactation and beyond (Hannigan and Pilati, 1991, Hannigan et al., 1993, Vorhees, 1989). Importantly, lower weights in PAE offspring have also been observed in cross-fostering studies (Abel and Dintcheff, 1978, Vorhees, 1989), indicating that retarded-growth is a result of the effects of alcohol on development rather than an indirect effect of deficits in postnatal maternal care. Indeed, a more recent study investigating maternal care and vocalizations reported no differences in maternal behaviour between alcohol and non-alcohol consuming dams (Marino et al., 2002). With that said, we cannot completely rule out potential issues with maternal behaviour as other studies have suggested that maternal behaviour may be compromised as a consequence of PAE offspring-dam interactions. For the most part, lower weights in PAE appear linked to fetal nutrient deficiencies and have been well-characterized (Abel and Dintcheff, 1978, Weinberg, 1985). The relationship between PAE-related nutritional deficiencies and persistent long-term cognitive deficits is less clear, however. In humans, lower birth weights unrelated to PAE are linked to a number of short and long-term developmental cognitive difficulties (Leitner et al., 2000, Hack et al., 1995). For example, the longitudinal study by Hack and colleagues (1995), which eliminated those children diagnosed with mental retardation or cerebral palsy, showed that although aetiology was largely unknown and effects varied widely, most low-birth weight children had some level of cognitive and attention impairment. Moreover, in some cases impairments carried over into adolescence. Although arguably nutritional deficiencies related to PAE are unique, in part owing to alcohol-induced metabolic changes (Weinberg, 1985), there is the suggestion that the isocaloric control, the pair-fed treatment, may also be susceptible to adverse nutritional effects on systems related to cognition. Iron deficiencies may offer a potential link between nutrition deficiencies, PAE, and cognition. Rufer and colleagues (2012) have shown that iron deficiencies, a relatively common nutritional deficit in pregnant women, produce a synergistic effect in a third trimester-equivalent model of PAE in rats. The consequences included abnormal brain development associated with cognitive dysfunction that persisted even after the iron deficiency was rectified. Understanding the influence of interacting factors outside of the teratogenic effects of prenatal alcohol will certainly aid in characterizing the range of deficits. Nevertheless, growth-retardation is not an inevitable

outcome found following moderate PAE, nor is it always the case for the pair-fed treatment group. As such, although it may certainly factor into the outcomes of PAE, it would be prudent to consider additional factors as well.

Adolescent CMS selectively increased basal corticosterone (CORT) levels

HPA function is sensitive to the deleterious effects of PAE resulting in dysregulation of the stress response system. For instance, PAE may increase HPA activation, as well as delay recovery to basal levels following stress; evidence of altered drive and deficits in feedback regulation. This is true in humans, as well as animal models of FASD. For example, Jacobson and colleagues (1999) reported increased CORT (cortisol) levels following blood withdrawal in infants of mothers with high alcohol abuse during pregnancy.

In the current study, a 5-day regimen of adolescent CMS produced an increase in basal corticosterone levels in control and pair-fed treatment groups relative to their non-stressed counterparts. However, we did not see a similar increase in the PAE animals, indicating atypical HPA function or reactivity in these animals. The absence of a stress-related change in basal corticosterone levels following PAE has been reported previously by our laboratory following a 10-day CMS regimen in adulthood, although in this latter study the pair-fed males also failed to show elevated CMS- related CORT levels (Uban et al., 2013).

In humans, chronic stress is more likely to produce enduring effects if experienced during periods of what can be considered increased vulnerability that includes early perinatal development and adolescence (for a review see Charmandari (2003)). Briefly, many long-term detrimental effects of stress can be linked to stress-induced alterations in endocrine systems because of chronic changes in circulating glucocorticoids, such as cortisol in humans and corticosterone in rats, with glucocorticoids themselves being regulated by the hypothalamic-pituitary-adrenal (HPA) axis. As in adulthood, chronic stress in adolescence potentially produces a host of neurobiological changes that include decreased neurogenesis (Barha et al., 2011, McCormick et al., 2012), changes in neuron morphology and connectivity (Liston and Gan, 2011), and neuronal atrophy (Chocyk et al., 2013). These alterations, when occurring during vulnerable periods, lead to organizational changes in brain development that manifests as aberrant or abnormal behaviour

Interestingly, the literature shows mixed outcomes of early adversity on basal cortisol in humans, with some studies reporting increased basal levels and others reporting decreased basal levels. What is more, negative or adverse outcomes related to dysregulation of the HPA system have been attributed to both increases, as well as decreases in cortisol. For example, Maldonado (2008) suggested a link between stress- induced HPA dysregulation and cognitive performance based on findings that showed children (ages 9 -12) with a high perceived daily stress level had impairments in cognitive performance as well as lower morning basal cortisol levels relative to children with low perceived daily stress levels. These alterations, when occurring during vulnerable periods, lead to organizational changes in brain development that manifests as aberrant or abnormal behaviour. For example, hypercortisolism in adolescence and adulthood is thought to be linked to over activation and representation of amygdalar input, thereby producing hyper-vigilance and perhaps even leading to the development of psychiatric disorders such as depression (Hellemans et al.,

2008). Thus, it would appear that dysregulation in HPA function, regardless of direction, has the potential to produce enduring changes in the developing brain that may be linked to subsequent cognitive deficits. Understanding, the exact influence of these hormonal changes will be important for future studies.

PAE and CMS interact to increase sensitivity

The T-maze represented the first experience in a reward-related learning environment. Although provided with a maximum of nine days of testing following habituation and training, all groups showed a tendency towards delayed learning relative to control animals. Again, it should be noted that due to the exclusion of four PAE animals (as well as one of the controls from the CMS condition) the T-maze and all subsequent test results represent those PAE animals at the higher end of functioning in this group. Although unfortunate, this is not atypical. Reyes and colleagues (1989) report that higher doses of PAE resulted in half of the treated animals in that experiment not reaching criterion in the radial arm maze. In the current study, the impact of PAE was not as extreme with, 20% rather than 50%, of the animals failing to learn the task within the provided time window. This is likely due, at least in part, to differences in extent of alcohol exposure. The current exclusion is important to keep in mind, as owing to the wide range of deficits along the spectrum this study likely represents the more subtle, yet relentless effects of moderate prenatal alcohol exposure.

PAE selectively produced attention deficits but these deficits were not associated with overall decreased performance in the 5-CSRTT

During acquisition of the 5-CSRTT, PAE- Non CMS accuracy and trials to criterion for each of the stimulus duration phases was comparable to that of the Con- Non CMS animals. However, although all treatment groups showed decreases in performance with transitions to progressively shorter stimulus durations, PAE animals showed decreased performance earlier in training relative to the control and Paired animals. Interestingly, reduced accuracy was not related to processing speed or motivation of the animals. All animals showed reduced latencies to make a correct nose-poke, a measure of processing speed, as well as reduced latencies to collect a reward, a measure of motivation, in these transitions. We also noted that, during acquisition perseverations, were increased in PAE and PAE- CMS animals, although only prior to the final transition phase in latter case. However, PAE animals exposed to adolescent stress, and to a lesser extent Non CMS PAE as well, also showed increased omissions, indicating reduced proficiency in maintaining sustained or focused attention. Therefore, deficits in processes of selective attention likely accounted for at least part of the reduced accuracy of PAE animals (Robbins, 2002). The unmasking of deficits with increased task complexity is compatible with findings in previous human (Burden et al., 2005, Connor et al., 2000) and animal model (Driscoll et al., 1990) studies of PAE.

We should note here that, although attention deficits are cited in numerous reports of PAE-related deficits in cognitive development in humans, there have been a few reports showing no deficits in sustained attention linked to PAE (Boyd et al., 1991). Importantly, this latter study investigated attention in preschool children, whereas the current study examined cognitive performance in adulthood, making age an important differentiating factor between

studies. Furthermore, it is possible that attentional deficits would only be dissociable once the area(s) that subserve this function, namely the PFC, reaches maturation. Indeed, it is suggested that the PFC is one of the later developing brain regions, not reaching full maturation patterns of connectivity until at least the mid 20s in humans (Sowell et al., 1999). As a reminder, early testing may not fully capture the extent of impairments that require maturation before being fully recognized or observable.

The effects of adolescent CMS are prenatal treatment-dependent

The current findings showing that the impact of five days of adolescent CMS is persistent and has the capacity to hamper learning in adulthood is quite remarkable. This supports adolescence as a unique period of increased vulnerability to environmental stressors as the impact of adult stress does not appear to be as profound. For instance, in studies by both McEwen and Sapolsky (1995) and Sousa (2000), chronic stress in adulthood did not produce long-term cognitive impairments. Nonetheless, our data is keeping with the few studies that been done on the consequences of adolescent social stress and adult cognition (McCormick et al., 2012). Importantly, the current study is in keeping with the notion that adverse environments, adolescent CMS in this case, amplify PAE-related deficits, as well as producing long-term deficits in control animals. Indeed, we show that the effects of CMS on PAE animals are unique. For instance, the current 5-day CMS regimen produced impairments in PAE, not only during the individual transitions, as was the case with their Non CMS counterparts, but also in acquisition as PAE- CMS animals required a substantially greater number of trials to reach the final training criterion than any other treatment group. However, acquisition deficits were seen only during the more taxing phases of acquisition, when stimulus duration was substantially shortened relative to the initial training sessions. This finding related to the interaction of PAE and postnatal adversity is again in line with the human literature. A novel study in humans by Henry, Sloane, and Black-Pond (2007), reported lowered cognitive function in children who experienced both alcohol exposure *in utero* as well as trauma such as abuse and neglect. It is appreciated that 5 days of chronic mild stress may not necessarily mimic cases of excessive neglect and abuse in humans. Nonetheless, the principles of both cases are shared in that they represent augmented effects of the postnatal environment on an already compromised brain because of PAE.

Acute Challenges Unmasked PAE and CMS-related deficits

Increasing the required hold time (enhanced ITI) is a means to challenge inhibitory control, a measure of impulsivity but also executive function and sustained attention. On the other hand, introducing a distracter, such as the random tone distracter in the current study, allows for the assessment of selective attention or ability to ignore irrelevant stimuli. Here we show a general decrease in accuracy across groups in response to the increased ITI. We also found increased premature responses, particularly in control animals in the CMS condition. Interestingly, we found contrasting effects during the acute challenges, with premature responses increased and perseverations decreased in response to the ITI challenge. This is quite interesting as, along with the similar contrasts with premature and perseveration in the acquisition phase; it highlights dissociable effects of PAE and the interaction with CMS on select aspects of attentional control. Moreover, as these two aspects of attentional control are

also dissociable in relation to the serotonergic system, with each selectively influenced by activation of specific receptor subtypes (Carli et al., 2006), the findings suggest that PAE may selectively affect specific circuitry.

Thus, it would appear that adolescent CMS might selectively affect aspects of the task linked to impulsivity. This type of distinction could be instrumental in providing insight into individual variations following PAE in humans as well.

The tone distracter on the other hand, did not appear to challenge most of the animals to the same extent as the ITI challenge, yet it had greater effects on PAE than controls. PAE animals showed increased perseverations relative to Con - Non CMS animals in response to the tone challenge, suggesting greater attention deficits. This is consistent with findings in clinical studies of FASD, where attention and distractibility are well-documented (Shaywitz et al., 1981, Aronson et al., 1985, Connor et al., 1999).

PAE animals showed a unique cognitive profile, allowing us to dissociate characteristics of PAE from those of Pairfed effects

A novel finding in the current study is that the PAE animals showed a unique cognitive profile, allowing us to dissociate characteristics of PAE from those of pair-feeding effects. Unlike the Pairfed animals that showed little impairment in the transitions during acquisition, PAE deficits in attention during acquisition were observable at the transition phases. The ability to dissociate between PAE and the Pairfed treatment, with the latter utilized to separate nutritional effects of ethanol from its direct effects has posed a particular challenge in the field of alcohol exposure as the Pairfed treatment has consistently been shown to be a treatment in of itself (Weinberg, 1984, Glavas et al., 2007). There are a number of potential reasons for the Pairfed effects. For example, despite the fact that experimental diets are formulated to provide optimal nutrition during pregnancy, underfeeding constitutes a mild prenatal stressor, which can itself impact offspring behavioral and physiological responses (Kwong et al., 2000).

Varying doses of d-amphetamine produced differential effects in the 5-CSRTT dependent on pre and postnatal environment and cognitive process

In the current study, we incorporated the administration of systemic *d*-amphetamine to assess potential alterations treatment and adolescent alter attention processes because of PAE and adolescent CMS. In response to the AMPH challenge, controls animals showed no real changes in accuracy, latency (to respond or to collect the reward), nor in number of initiated trials over the course of the challenge days. They did however; show an increase in premature responses as predicted (Robbins, 2002, Cole and Robbins, 1987), as well as a slight increase in percent of omissions (from 2% with saline injections to about 10% with the highest, 1.0 mg/kg, dose of AMPH). Controls exposed to CMS as adolescents showed elevated premature responses as well as omissions in comparison to the non-stressed controls. In addition, the single 5-day regimen of adolescent CMS produced a significant dose-related decline in accuracy in control animals alone, as well as an increase in collection latency in response to the 1.0 mg/kg AMPH challenge. Of interest, although 0.1 to 0.4 mg/kg of AMPH has been shown to enhance some cognitive processes in non-stressed rats,

such as sustained attention (Grilly and Loveland, 2001), the interactive effects of PAE and CMS in response to amphetamine did not show a benefit to sustained attention. Indeed PAE-CMS rats showed a unique profile that differentiated the PAECMS group from all others. In fact, PAE-CMS showed no observable effects from the varying doses of d-amphetamine. Moreover, we did not see a dose-related increase in impulsivity, nor were there any apparent modifications to selective attention. Rather, along with the blunted or dampened effects of amphetamine, we found a significant decrease in the number of trials initiated by the PAE-CMS animals. This blunted response to amphetamine is indicative of dopaminergic dysregulation and has been shown following other models of early life insult as well. For instance, amongst others, Choy and van den Buuse (2008) reported a dulled or blunted behavioural response to an amphetamine challenge in adult animals exposed to 24 hours of maternal deprivation on postnatal day 9. However, in contrast to our findings, the dulled amphetamine response in the Choy and van den Buuse study occurred with or without the addition of a 2-week corticosterone treatment in late adolescence (Choy and van den Buuse, 2008). Nonetheless, the researchers have also demonstrated abnormalities in DA function using a “two-hit” stress paradigm in which subjects are exposed to a neonatal and adolescent adverse stress regimen (Choy et al., 2009). Moreover, adolescents exposed to a 28-day regimen of social stressors alone have shown a dulled response to later AMPH challenges in otherwise intact animals (Kabbaj et al., 2002), suggesting the adolescent DA system may be especially vulnerable to insult. Although we incorporated a CMS model of unpredictable stress using a combination of social and psychological, but not physical stressors, the findings of Kabbaj and colleagues (2002) support our data, at least in part. The one caveat is that although PAE animals in the CMS condition in the current study did indeed show a dulled response to AMPH, the control animals in the CMS condition did not. However, due to differences in the duration of the stress regimen between studies, the discrepancy might indicate a heightened stress sensitivity in PAE animals as the 5-day regimen in the current study was sufficient to bring about changes in PAE but not control animals. At any rate, the differential responses of control and PAE subjects highlights the potential negative impact of early environmental factors on later interactions and susceptibility.

Conclusions

We assessed the outcome of PAE, adolescent CMS, and their interaction using a novel, comprehensive analysis of attentional processes recorded during acquisition and multiple test challenges thought to disrupt selective attentional processes (Amitai and Markou, 2011) in the 5 CSRTT. Overall, the current findings support and extend findings from clinical studies that indicate sustained attention may be especially vulnerable to the effects of *in utero* alcohol exposure, and in the case of visual attention at least, reportedly as a consequence of target stimuli insensitivity (Coles et al., 2002). In addition, the findings indicate that the environment, at least during adolescence, may be an important predictor of adult EF outcome following PAE, producing unique behavioural profiles that may help to explain the variability often reported following PAE, both in human and in animal models. Importantly, we have also been able to show PAE-related deficits linked to attention separate from those aspects of performance linked to impulsivity or response control, such as shown by premature and perseverative responses, as noted by Robbins (2002). We

present the novel finding that PAE animals show a unique cognitive profile, allowing us to dissociate characteristics of PAE from those of pair-feeding effects. Moreover, unmasking of deficits under various contexts, highlights potential limitations of studies that have reported the absence of long-term effects of moderate PAE (Tattoli et al., 2001), as it may be due to the restrictions based on the use of selective tests under a single paradigm.

Finally, these results help to provide direction in elucidating underlying mechanisms of increased susceptibility to postnatal adversity in vulnerable already compromised individuals, such as in the case of PAE. One of the potential explanations for the suspected increased sensitivity of PAE to stressors is that the effects of stress are compounded by an already dysregulated stress response system. Indeed, we would agree with Moghaddam's (2002) argument that contrary to popular theories, comorbidity between stress and other neurological disorders (PAE in this case) is perpetuated by a dysfunctional stress response rather than stress *per se*. There is at least some support for this notion in clinical PAE literature as well as it has been noted that problems often emerge under stress in vulnerable individual with FASD (Olson et al., 1998). Mechanistically, a recent report using a mouse model of increased psychiatric disorder vulnerability and adolescent stress, adds some potential insight into the current results and provides an exciting hypothesised influence of epigenetic mechanisms driven by the interaction of glucocorticoids and dopamine (DA) dysregulation (Niwa et al., 2013). Importantly, DA dysregulation following PAE is not novel, with PAE in some cases producing a down-regulation of DA activity thought to underlie at least some cognitive deficits. In addition, DA dysregulation has been linked to growth deficiencies in Pairfed and PAE offspring (Sobrian et al., 2005). The implications of DA and HPA dysregulation and their potential interaction in the current study are of great interest and importance in understanding the potential synergistic effects of the environment on developmental processes. Further research is needed however, to elucidate the underlying mechanism and to determine the full implications and mitigating effects of the postnatal environment.

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Abbreviations

AMPH	amphetamine Con-control
CMS	chronic mild stress
CORT	corticosterone (rat); cortisol (human)
DA	dopamine
EF	executive function
(5-CSRTT)	Five-choice serial reaction time task
FASD	fetal alcohol spectrum disorder

HPA	hypothalamic-pituitary-adrenal
ITI	intertrial interval
PAE	prenatal alcohol exposure
PF	pair-fed
PND	postnatal day
S	Seconds

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Table 1
Maternal Gestational Body Weight

The table depicts variations across dams during gestation and lactation. Multiple comparisons revealed that PAE dams had a reduced percent gain over the gestation phase ($P = 0.03$), whereas PF dams showed a trend toward reduced gain ($P = 0.108$). * significant, $P < 0.05$ compared to Con; # trend, $P < 0.11$ compared to Con. Values are means $\pm$ SEM.

Treatment	Body Wt (G1) (SE)	Body Wt (G21) (SE)	Gain (%) (SE)	Gestation Days Mean(+/-)
Control	256 (4.1)	389 (9.9)	51.1(2.6)	23.1 (.32)
Pairfed (PF)	248 (5.0)	358 (10.5) *	44.3 (2.3)	23.3 (.45)
Alcohol (PAE)	248 (6.7)	350 (9.5) *	41.6 (3.6) *	23.3 (.69)

* indicates a significance at $P < 0.05$.

Table 2

Summary of outcomes from the learning and challenge phases of the 5-CSRTT

Treatment Condition	Control		Pairfed		PAE	
	Non CMS	CMS	Non CMS	CMS	Non CMS	CMS
Learning- Sessions	-	-	-	-	-	↑*a ⁶ ,b ⁶
Accuracy	-	↓*	-	↓*	-	↓*c,a
Persev.	-	-	-	-	↑*a ³ ,b ³	↓c ^{2,3,4} ;↑d;↑c ⁵
Premature	↑d	↑d	↑d	↑d	↑d	-
Omission	↑d ³	↑d ¹	-	-	-	↑d ^{2,4,7}
Correct Latency	-	↑*	↓a	-	↓a	-
Collect Latency	↓	↓	↓	↓	↓	↓
Challenge ITI- Accuracy	↓	↓*	↓	↓*	↓	↓*
Persev.	-	↓y	↓y	↓y	↓y↑a	↓y↑a
Omission	-	↑*	-	-	-	-
Premature	↑y	↑y,*,b,c	↑y	↑y	↑y	↑y
Correct Latency	↓y	-	↓y	↓	↓y	-
Collect Latency	-	-	-	-	-	↓a
Challenge Tone-Accuracy	-	-	-	-	-	-
Persev.	↓y	↓y	↓y	↓y	↑a	-
Omission	-	-	↑a	-	-	-
Premature	-	↓y,b	↓y	↓y	-	-
Correct Latency	↓y	-	-	-	-	-
Collect Latency	-	-	-	-	-	-
AMPH- Accuracy	-	↓y	-	-	-	-
Premature	-	↑*	↓a,b	-	-	↑*
Omission	-	↑*	-	-	↑a,c	↓*,a
Correct Latency	-	-	-	-	-	-
Collect Latency	-	-	↑a	↓a	↑*a,c	-
Trials	↑	↑	↑	↑	↑	↓a
Valid trials	-	-	-	-	-	↓a

All reduced/deported differences are significant at $P < 0.05$; * = diff. from Non CMS counterpart; a = diff from control; b = diff from PF; c = diff from PAE; d = diff. in transition; y = diff, in transition; subscript numbers (1 to 7) = transitions 20 s to 1 s, respectively.