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Effects of Neonatal Methamphetamine and Stress on Brain Monoamines and Corticosterone in Prewanling Rats

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Abstract

Neonatal exposure to methamphetamine (MA) and developmental chronic stress significantly alter neurodevelopmental profiles that show a variety of long-term physiological and behavioral effects. In the current experiment, Sprague-Dawley rats were exposed to one of two housing conditions along with MA. Rats were given 0 (saline), 5, or 7.5 mg/kg MA, four times per day from postnatal day (P)11 to 15 or P11 to 20. Half of the litters were reared in cages with standard bedding and half with no bedding. Separate litters were assessed at P15 or P20 for organ weights (adrenals, spleen, thymus); corticosterone; and monoamine assessments (dopamine, serotonin, norepinephrine) and their metabolites within the neostriatum, hippocampus, and prefrontal cortex. Findings show neonatal MA altered mono-amines, corticosterone, and organ characteristics alone, and as a function of developmental age and stress compared with controls. These alterations may in part be responsible for MA and early life stress-induced long-term learning and memory deficits.

Keywords

Methamphetamine; Barren cage rearing; Corticosterone; Dopamine; Serotonin; Norepinephrine

Introduction

Children from lower socioeconomic status (SES) households often have elevated stress from multiple risk factors that include but are not limited to substandard housing and poor neighborhood quality (Evans and Kantrowitz 2002; Evans and Kim 2010). These environmental situations serve as a chronic stressor and studies reveal that low SES children experience more stressful life events compared with children from higher SES backgrounds (Attar et al. 1994). Chronic stress, such as deprived housing conditions, produces protracted

Compliance with Ethical Standards

Conflict Statement The authors declare no conflicts of interest.

activation of the hypothalamic-pituitary-adrenal (HPA) axis, causing an increase in the production of cortisol. Low SES-induced stress has been linked to increases in cortisol output and possible long-term CNS damage (Clearfield et al. 2014; Vliegenthart et al. 2016).

In rodent models of early life stress, activation of the HPA axis increases corticosterone (CORT) levels, as well as monoamine concentrations (Champagne et al. 2004; Zhang et al. 2005; Barbosa Neto et al. 2012; Masis-Calvo et al. 2013; Ohta et al. 2014). Although adrenal output of the HPA axis is normally blunted during the stress hyporesponsive period (Schoenfeld et al. 1980), this attenuation may only compensate up to a certain level of stress. In addition to the direct effects of stress on the HPA axis, variation in monoamine levels may provide a neuroanatomical substrate for stress-induced deficits seen in spatial and non-spatial learning and memory (Brunson et al. 2005; Masis-Calvo et al. 2013).

Chronic stress can be modeled in rodents by limiting the amount of nesting material. This impoverished environment induces significant maternal stress, and this stress is associated with aberrant nurturing behaviors, such as decreased licking and grooming and shortened nursing bouts (Baram et al. 2012; Ivy et al. 2008). These early life stress situations also alter dopamine (DA) and serotonin (5-HT) concentrations (Masis-Calvo et al. 2013; Ohta et al. 2014). When dams and pups are returned to normal bedding conditions, maternal behavior and monoamine levels rapidly returns to normal (Champagne et al. 2004; Ivy et al. 2008; Ohta et al. 2014).

In addition to stress, methamphetamine (MA) use is associated with low SES on multiple predictor variables such as marital status (single-parent households), employment status, and lower educational attainment (Diaz et al. 2014; Wouldes et al. 2014). Pregnant women seeking treatment for MA dependence at federally funded treatment centers rose from 8 % in 1994 to 24 % in 2006, surpassing rates for both non-pregnant women (12 %) and men (7 %; Terplan et al. 2009). MA is a potent psychostimulant that blocks DA reuptake (Sulzer et al. 2005). Children exposed to intrauterine MA show changes in cellular metabolism in the striatum and frontal lobe (Smith et al. 2001; Chang et al. 2009) and significant volume reductions in the putamen, caudate nucleus, globus pallidus, and hippocampus (Derauf et al. 2009; Smith et al. 2015) that correlate with attention, verbal memory, and reaction time deficits (Chang et al. 2004). Nearly half (42 %) of women who reported MA use during pregnancy continued using during the third trimester (Della Grotta et al. 2010). In animal models, the preweaning period of rat brain development [postnatal day (P)1–20] is approximately equivalent to human development during the second half of gestation (Rice and Barone 2000; Workman et al. 2013), and MA exposure during this time induces changes to developing monoamine systems (for review, see Jablonski et al. 2016a). For example, short-term increases in striatal dihydroxyphenylacetic acid (DOPAC) and the DOPAC/DA ratio and 5-HT reductions in hippocampus and striatum were seen shortly after MA treatment (10 mg/kg, four times/day from P11 to 20; Schaefer et al. 2008; Grace et al. 2010a). Further, our lab has documented reliable long-term deficits in tasks that require the striatum and hippocampus following MA administration during this developmental window (for review, see Jablonski et al. 2016b). It is possible that monoamine changes in these brain regions contribute to the learning and memory impairments observed during adulthood.

Studies in children following prenatal MA exposure in the context of alterations in HPA axis function, find that MA exposure is associated with newborn neurobehavioral patterns of increased stress and cortisol (Smith et al. 2008; LaGasse et al. 2011; Kirlic et al. 2013; Twomey et al. 2013; Zuloaga et al. 2015). In neonatal rats, MA administration increases CORT release (Schaefer et al. 2006, 2008, 2010; Williams et al. 2006; Grace et al. 2010a, b). When repeated MA administration was examined for four consecutive time windows from P1 to 19, with an acute dose on the fifth day, CORT release increased with age (Williams et al. 2006). In fact, CORT release after neonatal MA is greater than release in response to known stressors such as forced swim or isolation (Grace et al. 2008). Furthermore, MA alters the adrenal response to a forced swim stressor during adulthood (Williams et al. 2003). MA administration from P11 to 20 also results in short- and long-term alterations in the expression of HPA axis-associated peptides (Williams et al. 2000; Schaefer et al. 2006, 2008; Zuloaga et al. 2013).

Because early life stress and MA exposure induce changes in the stress response, neurochemistry, and behavior of the exposed animals (Vorhees et al. 2009; Graham et al. 2011), we examined the combined effect of chronic developmental stress and MA exposure on stress responses and neurochemistry. Monoamines and their metabolites were determined in the neostriatum, hippocampus, and prefrontal cortex at two ages (P15 and P20). These brain regions were selected based on their susceptibility to structural and functional abnormalities observed in both humans and animals (Jablonski et al. 2016b). We used a mild chronic stressor (barren cage housing) versus standard cage rearing (Gilles et al. 1996; Ivy et al. 2008) and two dose levels of MA (5.0 or 7.5 mg/kg/four times/day from P11 to 15 or P11 to 20).

Materials and Methods

Subjects

Rats were treated in accordance with protocols approved by the Institutional Animal Care and Use Committee. Animals were maintained in an AAALAC International-accredited vivarium with controlled temperature (19 ± 1 °C) and humidity (50 ± 10 %) and a fixed light-dark cycle (14:10 h, lights on at 0600 hours), and all care and use conformed to the NIH Guide on the Care and Use of Laboratory Animals in Research. Rats had ad libitum access to NIH-07 rat chow and reverse osmosis filtered, UV sterilized water. Following a minimum of 1 week of habituation to the vivarium, male and nulliparous female Sprague-Dawley CD (IGS) rats (strain #001, Charles River Laboratories, Raleigh, NC) were housed together for breeding. Female rats were examined for a sperm plug each day and, if found, that day was designated as embryonic day 0 (E0). On E1, gravid females were individually housed in polycarbonate cages (46 cm $\times$ 24 cm $\times$ 20 cm) containing woodchip bedding and a stainless steel hut as enrichment (Vorhees et al. 2008). Date of birth was designated as P0. On P1, litters were culled to eight male pups. If a litter had only six or seven males, one or two males from another litter with the same date of birth were fostered into the litter to attain a uniform litter size. Fostering occurred in 18 out of 36 litters. Of the 18 litters that had pups added, 13 received one pup (36.1 %) and 5 received two pups (13.9 %). On P4, pups were ear-punched for identification. Only males were used because of the number of animals

required per litter, and we have found few, if any, sex-dependent MA effects (Vorhees et al. 2000, 2007). The split litter design was used because we have shown that regardless of litter composition (i.e., all animals in a litter receiving the same dose of MA or animals in a litter receiving different drug exposures) the long-term effects on behavior are consistent (Jablonski et al. 2016b). Furthermore, the split litter design provides some control over the metagenome and microbiome (Stappenbeck and Virgin 2016). While McDonnell-Dowling and Kelly (2015) suggest the possibility of inadvertent MA exposure to the dam or control pups via MA-treated pups' excrement, there are no data to support this speculation.

Rearing and Methamphetamine Treatment

Thirty-six litters were enrolled but 3 litters were not be used (Table 1); hence, data analyzed were from 33 litters (final $n = 3-8$ per litter; see Table 2 and Figs. 1, 2, 3, and 4 for final group sizes). Half the litters were assigned to standard and half to barren-caged housing. Differential housing began on P4 and continued until sacrifice (P15 or P20). Standard-caged housing was as described above. For barren-caged housing, the woodchip bedding and stainless steel hut were replaced with a single paper towel, as described in Gilles et al. (1996), with modification (Graham et al. 2011). Cages and paper towels were changed each day.

Pups were treated with (+)-MA HCl (MA, Sigma-Aldrich Co., St. Louis, MO, expressed as free base and >95 % pure) or saline (SAL; 0.9 % NaCl). Within each litter ($n = 8$ per litter for consistency with previous studies), subsets of pups were given one of the following: three pups were given 5.0 mg/kg MA, three pups were given 7.5 mg/kg MA, one pup was given SAL, and one was not used in this study. The choice of MA doses was made by extrapolating from the dose that we demonstrated cause reliable long-term learning and memory deficits (10 mg/kg). We did not include a 10 mg/kg MA dose in the current experiment since findings from a pilot experiment showed that 10 mg/kg MA combined with barren cage housing was not well-tolerated by pups. Rats were assigned to groups using a random number table. Beginning on P11, drugs were administered every 2 h for a total of 4 s.c. injections per day (dosing volume of 3 ml/kg in saline) and ended at either P15 or P20. Because the neonatal period in rats is analogous to the third-trimester brain development in humans (Clancy et al. 2001, 2007), the drug was delivered to the pups. Therefore, the neonatal rat models a human fetus, rather than a human infant. We have shown no differences in CORT levels between saline-injected and weighed-only controls, suggesting that injection stress alone does not influence CORT levels at this age (Schaefer et al. 2010). No more than one littermate was assigned to any given experimental condition [age of assessment (P15, P20) $\times$ treatment (MA5, MA7.5, SAL) $\times$ rearing (standard, barren)]. Body weights were recorded prior to each drug treatment.

Pups were sacrificed 60 min following the final drug treatment on P15 or P20. Blood was collected in polyethylene tubes (12 $\times$ 75 mm) containing 0.05 ml of 2 % EDTA. For monoamines, neostriatum (caudate-putamen), hippocampus, and prefrontal cortex were dissected over ice with the aid of a 1-mm brain block (Zivic-Miller, Pittsburgh, PA). Adrenal, spleen, and thymus were obtained and weighed. Body weights were recorded. Samples were stored at -80°C until assayed.

Corticosterone Assessment

Trunk blood was centrifuged at 4 °C for 25 min (1300 relative centrifugal force (RCF)) and samples stored at –80 °C until assayed. CORT was assayed using an EIA kit according to manufacturer instructions (Immunodiagnostic Systems Inc., Fountain Hills, AZ). Plasma was diluted 3:1 in provided buffer and samples were run in duplicate. Outliers were removed if CORT values were ± 3.0 standard deviations from the group mean.

Neurochemical Analyses

Tissue concentrations of DA, DOPAC, homovanillic acid (HVA), norepinephrine (NE), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in the neostriatum, hippocampus, and prefrontal cortex were quantified via high performance liquid chromatography with electrochemical detection (as described in Graham et al. 2011). Frozen samples (–80 °C) were weighed, thawed, and sonicated in proper volumes of 0.1 N perchloric acid (Fisher Scientific, Pittsburgh, PA) and centrifuged at 4 °C for 14 min (13,000 RCF). The supernatant was injected onto a LC-18 column (150 $\times$ 4.6 mm, 3 μ m; Supelco Supelcosil™; Sigma-Aldrich Co., St. Louis, MO), connected to a 717plus autosampler (Waters Corp., Milford, MA), ESA 584 pump (ESA, Inc., Chelmsford, MA), and ESA electrochemical Coulochem detector. The potential settings were –150 mV for E1, +250 mV for E2, and a guard cell potential of +350 mV. The MD-TM mobile phase (ESA, Inc.) consisted of 75 mM sodium dihydrogen phosphate monohydrate, 1.7 mM 1-octanesulfonic acid sodium salt, 100 μ l/l triethylamine, 25 μ M EDTA, and 10 % acetonitrile (final pH of 3.0). Samples were processed at 28 °C with a pump flow rate of 0.7 ml/min. Quantification of the analytes was determined on the basis of known standards (Sigma-Aldrich Co., St. Louis, MO) prepared in 0.1 N perchloric acid. All neurotransmitters were run on a single chromatogram. Outliers were removed if tissue concentrations were ± 3.0 standard deviations from the group mean. Each age $\times$ treatment $\times$ rearing condition had different final sample sizes (with an average of 6–8, due to sample loss or technical malfunctions (see Table 2 and Figs. 2, 3, and 4 for group sizes)).

Statistical Analyses

Monoamine concentrations, body weight, and organ weights were analyzed using mixed linear factorial analysis of variance (ANOVA; Proc Mixed, SAS v9.3, SAS Institute, Cary, NC) with Kenward-Rogers degrees of freedom. Factors were treatment (SAL, MA5, and MA7.5) and rearing (standard, barren), and these were analyzed separately at each age (P15, P20). For body weights, the first weight taken on each day of treatment was used for analysis and day was a repeated measure factor. In order to account for litter effects, litter was a randomized block factor in ANOVA models. Significant interactions were further analyzed using slice-effect ANOVAs within Proc Mixed. Mortality was analyzed using Fisher's test for uncorrelated proportions. Significance was set at $p = 0.05$ and F ratios are only shown for main effects of treatment and rearing or their interaction. Data are presented as least square (LS) means $\pm$ SEM.

Results

Mortality

Mortality data are shown in Table 1. Fisher's test for uncorrelated proportions showed that at P15, animals reared in barren cages given either dose of MA showed increased mortality compared with MA animals in standard cages. At P20, barren-caged housing increased mortality in animals administered the low MA dose, but not the high MA dose compared with animals given MA in standard housing. At both ages, the increased mortality in MA5 and MA7.5 barren-housed animals compared with barren-housed SAL animals was significant ($p < 0.04$; one-tailed). At both dose levels, MA treatment led to a significant increase in mortality when combined with barren cage housing. Barren cage housing alone did not significantly increase mortality. No other contrasts were significant.

Body Weights

P20 body weight was missing from one animal (group MA7.5/barren). Animals treated from P11 to 20 showed significant main effects of treatment ($F_{2,84} = 12.62$, $p < 0.0001$); rearing ($F_{1,84} = 10.49$, $p < 0.002$); and day ($p < 0.0002$). All animals showed increased weight gain from P11 to 20; however, a significant treatment $\times$ day interaction ($F_{18,722} = 6.16$, $p < 0.0002$) revealed that animals given MA5 and MA7.5 weighed less than SAL animals beginning on P14 and for the remainder of treatment (Fig. 1a). Additionally, a significant rearing $\times$ day interaction ($F_{9,720} = 6.10$, $p < 0.0002$) showed that barren animals weighed less than Standard animals beginning on P15 and for the remainder of treatment (Fig. 1b). No other interactions were significant. All main effects and interactions were similar for the P15 group.

Organ Weights

For organ weights, the number of animals used was as follows for P15, P20: SAL/standard = 8:8; SAL/barren = 7:8; MA5/standard = 8:9; MA5/barren = 8:10; MA7.5/standard = 8:8; MA7.5/barren = 7:8.

Adrenals—Organ weights (adrenal, spleen, and thymus) were expressed as a percentage of body weight at each age. For adrenals, at P15, no main effect of treatment or rearing was found; however, there was a significant treatment $\times$ rearing interaction in which MA7.5/standard animals (0.033 ± 0.002 %) had higher relative adrenal weights compared with MA7.5/barren animals (0.026 ± 0.002 %; $F_{2,27} = 7.41$, $p < 0.003$; Table 2). No main effects or interactions were observed for adrenal weights at P20.

Spleen—Relative splenic weight at P15 showed a main effect of treatment ($F_{2,26} = 23.28$, $p < 0.0001$), with MA5 and MA7.5 animals (0.36 ± 0.02 or 0.33 ± 0.02 % body weight, respectively) exhibiting reduced weights compared with SAL animals (0.48 ± 0.02 %; Table 2). No other differences were significant. No main effects or interactions were observed for splenic weights at P20.

Thymus—Relative weight on P15 showed a main effect of treatment ($F_{2,27} = 5.39$, $p < 0.011$) and rearing ($F_{1,14} = 27.22$, $p < 0.0002$), in which MA7.5 (0.26 ± 0.01 %), but not

MA5 (0.28 ± 0.01 %) animals, had smaller relative thymus weights compared with SAL animals (0.32 ± 0.01 %), and barren animals (0.23 ± 0.01 %) had smaller relative weights than standard animals (0.34 ± 0.01 %). A significant treatment $\times$ rearing interaction ($F_{2,27} = 6.02$, $p < 0.007$) revealed that barren MA5 and MA7.5 animals had decreased relative weights (0.21 ± 0.02 % and 0.20 ± 0.02 %, respectively) compared with SAL animals (0.30 ± 0.02 %). Additionally, the barren MA5 and MA7.5 animals had lower relative weights compared with their standard counterparts [MA5 (0.35 ± 0.02 %) and MA7.5 (0.33 ± 0.02 %; Table 2)].

On P20, relative thymus weights had a significant main effect of treatment ($F_{2,30} = 6.19$, $p < 0.006$), where MA5 and MA7.5 animals (0.29 ± 0.03 % or 0.29 ± 0.03 %, respectively) exhibited decreased relative weight compared with SAL animals (0.36 ± 0.03 %; Table 2). The rearing main effect and treatment $\times$ rearing interaction were not significant.

Corticosterone Assessment—On P15, there was a main effect of rearing ($F_{1,14} = 9.07$, $p < 0.001$); pups raised in barren cages showed elevated CORT levels compared with standard cage animals (13.1 ± 1.6 ng/ml vs. 6.4 ± 1.6 ng/ml). The treatment $\times$ rearing interaction showed a trend ($F_{2,24} = 2.82$, $p < 0.08$) (N per group: SAL/standard = 8, SAL/barren = 7; MA5/standard = 7, MA5/barren = 7, MA7.5/standard = 6, MA7.5/barren = 6).

On P20, CORT levels were affected by treatment ($F_{2,22} = 4.23$, $p < 0.03$), but not by rearing. For the treatment effect, MA5 animals had a significant increase in CORT levels compared with SAL animals (14.9 ± 1.9 vs. 8.5 ± 2.0 ng/ml); this was not seen in the MA7.5 animals. No effect of rearing or interaction was found (N per group: SAL/standard = 5, SAL/barren = 8; MA5/standard = 6, MA5/barren = 8, MA7.5/standard = 5, MA7.5/barren = 7).

Monoamine Measurements

The following summarizes the main effects and/or interactions among the monoamines (DA, 5-HT, and NE) and major metabolites (DOPAC, HVA, and 5-HIAA) within each brain region (neostriatum, hippocampus, and prefrontal cortex), for each age (P15, P20). Statistical analyses and group comparisons are presented in the captions for monoamines (Figs. 2, 3, and 4) and metabolites (Table 3).

Neostriatum—At P15, regardless of housing condition, MA (at one or both doses) decreased striatal DA (Fig. 2a), DOPAC, HVA, and 5-HIAA levels, but increased NE levels (Fig. 2c). Similarly, at P20, MA decreased DA (Fig. 2d), DOPAC, and 5-HIAA. No differences were noted in MA-treated animals for 5-HT at P15 (Fig. 2b) or P20 (Fig. 2e) or for NE at P20 (Fig. 2f). Irrespective of MA treatment, compared with standard housing, barren cage housing increased DOPAC levels at P15 but not at P20 and increased NE at P20 (Fig. 2f) but not at P15.

Hippocampus—Within the hippocampus, MA decreased 5-HT (Fig. 3b, e), 5-HIAA, and NE (Fig. 3c, f) at both P15 and P20, but not DA (P15: Fig. 3a), DOPAC, or HVA compared with SAL. Compared with standard housing, barren cage housing increased 5-HIAA levels at P15 and decreased DA levels at P20 (Fig. 3D), regardless of MA treatment. Barren cage

housing in combination with MA increased NE levels at P15 (Fig. 3c) and decreased DA levels at P20 (Fig. 3d) compared with standard reared animals given MA.

Prefrontal Cortex—At P15 and P20, MA decreased 5-HT (Fig. 4b, e), 5-HIAA, and NE (Fig. 4c, f) in the prefrontal cortex compared with SAL. A decrease in HVA was also seen at P15. No main effect of MA was observed for DA at either age (Fig. 4a, d). Barren cage housing increased 5-HT and 5-HIAA levels compared with standard housing, at both ages (Fig. 4b, e). Additionally, at P20, high-dose MA interacted with standard housing and increased DA compared with controls (Fig. 4d), low dose-MA, and their barren caged counterparts.

Discussion

This experiment examined whether MA administered during the human third trimester equivalent in rats in conjunction with standard or barren cage housing altered indices of stress and brain monoamines. Women of child-bearing age account for a substantial portion of MA users (SAMHSA 2014) and low SES serves as a chronic stressor linked to increases in cortisol reactivity (Clearfield et al. 2014). We included a deprived environmental rearing situation (barren cage housing) to model chronic stress since MA exposure is typically evident in areas of lower SES, including increased stress and deprived situations (Diaz et al. 2014; Woulides et al. 2014). Even though both MA and chronic stress are harmful, including in animal models, the extent to which they interact is unknown.

A decrease in body weight was evident during treatment for both MA groups, regardless of housing condition. In addition, barren cage housing itself decreased body weights, irrespective of drug treatment. We have consistently reported a decrease in body weight in MA-exposed offspring following various developmental MA treatment scenarios (Vorhees et al. 1994; Williams et al. 2000, 2003, 2004, 2006), and these reductions are similar to those described in humans (Oro and Dixon 1987; Smith et al. 2003). We have also shown a decrease in body weights in animals reared in barren cages (Graham et al. 2011; Vorhees et al. 2014). However, alteration in body weight is not predictive of MA-induced cognitive deficits (Vorhees et al. 1994, 2000, 2009). For example, MA from P1 to 10 induces greater body weight reductions compared with administration from P11 to 20 or P6 to 15; however, it is MA administration during the P11–20 and P6–15 time frames that produce the greatest cognitive deficits (Vorhees et al. 1994, 2009). In addition, MA given via multiple injections produces more severe cognitive impairment than the same dose with fewer number of injections, despite no difference in body weight among groups (Vorhees et al. 2000). Postnatal undernutrition has no effect on spatial learning (Campbell and Bedi 1989; Goodlett et al. 1986). Thus, body weight and nutrition factors do not contribute to the current findings.

We found an increase in mortality associated with MA treatment when combined with barren cage rearing. Other studies with developmental manganese and lead, along with barren cage housing, show similar effects (Graham et al. 2011; Vorhees et al. 2014). Even though postnatal MA exposure or barren cage housing alone does not lead to an increase in mortality, the present data suggest a combined effect of the two factors does. The mortality

rates reported here are consistent with those seen in adult animals after comparable MA exposure and chronic stress (Matuszewich and Yamamoto 2004). In adult animals, chronic stress enhances MA toxicity through enhanced hyperthermia (Broom and Yamamoto 2005; Tata et al. 2007). During neonatal MA exposure, however, hyperthermia is not induced in MA-treated rats (Cappon et al. 1997; Kokoshka et al. 2000). The lack of hyperthermia suggests an alternate mechanism is responsible for MA and stress-induced mortality during development.

On P15, barren housing increased CORT levels, regardless of treatment, and therefore demonstrates that this procedure is stressful at least during the first 5 days. On P20, the low, but not high, MA dose increased CORT irrespective of rearing condition. It is somewhat surprising that CORT was not increased on P15 by MA, nor was it increased following the high dose of MA at P20. We have shown CORT increases following single-day MA administration at different postnatal ages (Williams et al. 2000; Schaefer et al. 2006, 2010) and after 4–5 days of consecutive MA exposure (Williams et al. 2000, 2006; Schaefer et al. 2008), but no difference in CORT levels when assessed later at P30 (Schaefer et al. 2008). In the present experiment, CORT determinations were taken from pups 60 min after the last MA treatment on P15 or P20. We have shown that CORT elevations are greater 30 min after a single-dose of MA on P15, with smaller elevations 65 min later (Williams et al. 2006). Similarly, P11–15 MA leads to increased CORT 30 min after the final dose, but not 65 min later (Williams et al. 2006). This may account for the lack of an effect of barren housing on CORT at P20. Hence, time of sacrifice is critical. The present experiment is consistent with others showing an increase in CORT following barren cage housing as early as P11, but not later on P20 (Graham et al. 2011; Vorhees et al. 2014). Early life stress models, including limited bedding prior to P10, also lead to alterations in plasma CORT (Ivy et al. 2008; Moriceau et al. 2009a, b) that return to baseline later (Brunson et al. 2005).

We also tested the effects of MA and stress on organ weights associated with stress and the HPA axis. On P15 only, the high MA dose along with barren-cage housing decreased adrenal weights suggesting an overall combined effect. Neither MA nor stress alone influenced adrenal weights. Since MA alone did not induce a change in CORT levels (see above), but barren cage housing did, it is possible that barren cage housing, rather than MA, may have a greater effect on the stress system associated with the adrenals at P15. This effect may arise from the chronicity of the barren cage housing compared to MA administration. We have previously reported no change in adrenal weights with barren cage housing from P4–29 (Graham et al. 2011); however, others have reported that barren-cage rats display adrenal hypertrophy at P9 when the barren cage environment is started on P2 (Gilles et al. 1996; Avishai-Eliner et al. 2001).

On P15, splenic weights were decreased in both MA groups, regardless of housing condition. This effect also did not persist until P20. We have shown a reduction in spleen weights with barren-cage housing from P4 to 29 (Graham et al. 2013), but here, there was a change in spleen weights with a shorter barren cage rearing interval. Thus, while the impact of stress on the spleen is evident during adulthood (Spencer et al. 1991; Johnson et al. 2002; Pruett et al. 2007), its effects during development are more subtle over the short term.

During adulthood, long-term exposure to stressors causes involution of the thymus (Kioukia-Fougia et al. 2002). Here, both MA dose levels and barren cage housing decreased relative thymic weights at P15, replicating our previous findings in which we showed a similar reduction in thymic weights after developmental MA exposure (Williams et al. 2006). However, barren cage housing did not interact with the MA-induced decrease in thymic weights at P20. These data suggest that animals exposed to stress and/or MA early in development show transient effects on CORT-sensitive organs.

Selective effects of MA and barren cage housing on monoamines were evident later in development. For example, at P20, barren cage housing decreased hippocampal DA levels, with the effect observed predominately in both MA dose levels. A DA reduction after barren cage housing was also evident in the prefrontal cortex at P20 with the high MA dose. Particularly in the hippocampus, then, it may be that there is an increase in sensitivity of DA to barren cage stress after longer MA dosing periods (P11–20 vs. P11–15). We also found an increase in DOPAC, but not DA, in the neostriatum at P15 for barren cage animals, compared with those in standard rearing. Thus, while DA was decreased by the combination of MA and barren cage, the degree of interaction with monoamine metabolites was mixed.

Consistent with previous findings, MA affected 5-HT. At both ages, MA decreased 5-HT levels in the hippocampus and prefrontal cortex. A significant decrease in 5-HIAA was seen in all three brain regions at both ages. Similar to DA, it may be that long-lasting alterations to the 5-HT system persist beyond the MA treatment period, since some changes were reported out to P30 (Schaefer et al. 2008). With regard to chronic stress, an increase in 5-HT after barren-cage housing was evident in the PFC on P20. While there was no main effect of rearing cage, 5-HIAA was increased after chronic stress in combination with the high dose of MA. Barren cage housing also increased hippocampal 5-HIAA levels on P15. Dams characterized as low licking and grooming (as a measure of reduced maternal care) produce an increase in 5-HT turnover in the prefrontal cortex (Masis-Calvo et al. 2013). For 5-HT, then, barren cage housing may serve to increase, rather than decrease 5-HT levels. Prolonged maternal separation stress beginning on P2 also alters 5-HT receptor expression in pups at P7 and 14 (Ohta et al. 2014).

We found a decrease in NE levels by MA at both ages within the hippocampus and prefrontal cortex, similar to its effects on the 5-HT system. Why there was not a reduction by MA in 5-HT and NE within the neostriatum is unclear. Within the neostriatum, barren cage housing increased NE levels at P20 and a similar increase with barren cage housing was reported in the hypothalamus at P19 (Graham et al. 2011). The effects of barren cage housing on NE and the sympathetic nervous system may be important in light of the current findings. Early life stress, as modeled by maternal separation during early development, induces a sensitization of the sympathetic nervous system (Loria et al. 2013; Reho and Fisher 2015). For example, compared with controls, NE content is significantly increased in the spleen and adrenals of maternally separated offspring, and these rats display significantly greater increases in mean arterial pressure in response to NE administration, suggesting a sensitization of the sympathetic system by early life stress (Loria et al. 2013). In comparison to early life stress, MA also stimulates NE release from sympathetic nerve terminals in adult animals (Makisumi et al. 1998), and administration of 6-OHDA to neonatal rats causes

destruction of developing sympathetic axons (Yodlowski et al. 1984). Thus, developmental barren-cage stress and MA may produce similar alterations to the sympathetic nervous system and stress organs by alterations to NE signaling. MA during the neonatal period induces learning and memory impairments in hippocampal-dependent spatial tasks such as the Morris water maze (MWM). We have narrowed the sensitive exposure window of MA treatment to the overlapping periods of P6–16 and P11–20, whereas P1–10, P21–30, and later 10-day exposure periods are less effective or not effective at all at inducing deficits in the MWM (Williams et al. 2003; Vorhees et al. 2005, 2008, 2009). In fact, MA doses as low as 0.625 mg/kg (four times/day, from P11 to 20) result in MWM reference memory impairments (Williams et al. 2004; Vorhees et al. 2007). Because this exposure window is similar to the MA regimen used here, the fluctuation in monoamines and their metabolites may contribute to the P11–20 MA-induced spatial learning deficits. Even though the changes in DA and 5-HT do not reach the reductions seen during adulthood after MA, we have shown evidence for lasting alterations in the 5-HT and DA systems (Crawford et al. 2003; Schaefer et al. 2008; Graham et al. 2013). In the developing brain, 5-HT, for example, not only functions as a neurotransmitter but influences neuronal differentiation and synaptogenesis (Whitaker-Azmitia et al. 1996; Mazer et al. 1997), and early 5-HT changes have been associated with later learning and memory deficits (Mazer et al. 1997).

The present findings suggest that postnatal MA is more detrimental than chronic stress alone on the outcomes measured here. It is possible that longer barren cage exposure periods, procedures that produce an exaggerated stress response or measurements at later time points are required for understanding how MA and chronic stress interact during postnatal development. MA and stress may have influenced other neuronal systems not assessed here, in which MA is known to have an effect (e.g., BDNF, NGF; Skelton et al. 2007; Grace et al. 2008), suggesting developmental fluctuations in neurotrophic signaling may also be susceptible to chronic stress. Recent work suggests that developmental alterations in expression of synaptic plasticity proteins as a result of increased MA-induced oxidative stress may also play a role in the early effects of MA (Ramkissoon and Wells 2015, for review, see Jablonski et al. 2016b). The extent to which MA along with chronic stress influence these changes has yet to be examined.

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Fig. 1.

Body weights (g) were analyzed for the first dose of each day in those animals dosed from P11–20. Administration of MA (5 or 7.5 mg/kg) four times per day with an inter-dose interval of 2 h caused a decrease in body weight gain for both MA groups compared with SAL controls beginning on the fourth day of treatment (P14). **a** Main effect of treatment collapsed across rearing condition. **b** Main effect of rearing condition collapsed across treatment groups. Barren caged housing produced a decrease in body weight gain compared with standard housing beginning on P15. * $p < 0.05$ MA5 and MA7.5 versus SAL (**a**); barren versus standard cage rearing (**b**). Data are LS means $\pm$ SEM; $n = 7$ –9 per group $\times$ rearing condition

Fig. 2.

Neostriatal monoamine levels [pg/mg tissue], for dopamine (DA; **a, d**); serotonin (5-HT; **b, e**); and norepinephrine (NE; **c, f**) as a function of Treatment and Rearing on P15 and P20.

P15 DA: a There was a main effect of Treatment ($F_{2,27} = 4.32, p < 0.03$); in which MA7.5 animals ($p < 0.02$), but not MA5 animals, had lower DA levels compared with SAL animals. No other differences were significant for DA.

P15 5-HT: b There were no treatment or rearing main effects or interactions.

P15 NE: c There was a main effect of treatment ($F_{2,25} = 5.73, p < 0.01$) in which MA5 animals (158.6 ± 12.7 pg/mg) had higher NE levels compared with SAL (111.0 ± 12.3 pg/mg; $p < 0.01$) and MA7.5 animals (122.2 ± 12.7 pg/mg; $p = 0.05$).

P20 DA: d DA showed a main effect of treatment ($F_{2,31} = 8.07, p < 0.002$) in which MA7.5 (3222.0 ± 302.4 pg/mg; $p < 0.01$) and MA5 animals (4418.8 ± 288.4 pg/mg; $p < 0.003$) had lower DA levels compared with SAL animals (4761.1 ± 329.9 pg/mg) and MA7.5 animals had lower levels than MA5 animals. There was no interaction of treatment $\times$ rearing for DA.

P20 5-HT: e There was no significant effect of treatment ($p < 0.08$), rearing, or the interaction.

P20 NE: f There was no main effect of treatment ($p < 0.08$); however, there was a main effect of rearing ($F_{1,13} = 7.78, p < 0.02$). The barren animals had a significant increase in NE compared with standard animals (*inset*). No significant interaction was found.

Group sizes from *left to right*, P15 DA (standard/barren): SAL = 8:8, MA5 = 8:8, MA7.5 = 7 β :8; 5-HT: SAL = 8:7, MA5 = 8:8, MA7.5 = 6 β :7 β ; NE: SAL = 8:7 β , MA5 = 7 β :7 β , MA7.5 = 7 β :7 β ; P20 DA: SAL = 7 β :7 β , MA5 = 9:10, MA7.5 = 8:9; 5-HT: SAL = 8:8, MA5 = 9:10, MA7.5 = 7 β :9; NE: SAL = 8:7 β , MA5 = 9:9 β , MA7.5 = 8:9. β One outlier excluded because levels were >3 standard deviations from the group mean. *Inset* represents significant main effect. Data are LS means $\pm$ SEM * $p < .05$

Fig. 3.

Hippocampal monoamine levels [pg/mg tissue], for dopamine (DA; **a, d**); serotonin (5-HT; **b, e**); and norepinephrine (NE; **c, f**) as a function of treatment and rearing on P15 and P20.

P15 DA: a Hippocampal analyses showed no main effects or treatment $\times$ rearing interaction for DA. **P15 5-HT: b** There was a main effect of treatment on 5-HT ($F_{2,28} = 57.27$, $p < 0.0001$); in which MA5 and MA7.5 animals (77.5 ± 10.7 pg/mg and 69.0 ± 11.1 pg/mg, respectively), that did not differ from each other, had significantly lower 5-HT levels compared with SAL animals (209.8 ± 10.7 pg/mg, p 's < 0.0001). There was no rearing effect or treatment $\times$ rearing interaction for 5-HT. **P15 NE: c** A main effect of treatment for NE ($F_{2,27} = 92.56$, $p < 0.0001$) revealed that MA5 and MA7.5 animals (28.2 ± 5.4 pg/mg and 28.9 ± 5.8 pg/mg, respectively) had significantly lower NE levels compared with SAL animals (118.9 ± 5.6 pg/mg, p 's < 0.0001). There was no main effect of rearing, but a significant treatment $\times$ rearing interaction was observed ($F_{2,27} = 3.33$, $p = 0.05$). The MA7.5/barren animals (42.5 ± 8.2 pg/mg) had significantly higher NE levels compared with MA7.5/standard animals (15.4 ± 8.2 pg/mg, $p < 0.03$). No other differences were found. **P20 DA: d** There was a main effect of rearing on DA ($F_{1,11} = 4.93$, $p < 0.05$); Barren animals had significantly lower DA levels compared with standard animals (*inset*). A significant treatment $\times$ rearing interaction was seen ($F_{2,20} = 4.99$, $p < 0.02$), in which MA5/standard and MA7.5/standard animals had significantly higher DA compared with their barren-caged counterparts. Additionally, in barren animals, the MA5 and MA7.5 groups had significantly lower DA compared with SAL animals (p 's < 0.03). **P20 5-HT: e** A main effect of treatment on 5-HT ($F_{2,30} = 88.59$, $p < 0.0001$) showed that MA5 (128.9 ± 12.4 pg/mg) and MA7.5 animals (82.6 ± 13.0 pg/mg) had significantly lower 5-HT levels compared with SAL animals (279.1 ± 12.6 pg/mg, p 's < 0.0001), and MA7.5 animals had significantly lower 5-HT levels compared with MA5 animals ($p < 0.02$). No other effects for 5-HT were found. **P20 NE: f** A main effect of treatment ($F_{2,46} = 35.88$, $p < 0.0001$) showed that MA5 animals (72.9 ± 11.5 pg/mg) and MA7.5 animals (48.5 ± 11.8 pg/mg) had significantly lower NE levels compared with SAL animals (181.12 ± 11.8 pg/mg; p 's < 0.0001), with no difference between MA groups. No other significant effects were found. Group sizes from *left to right*, P15 DA: SAL = 6[♂]:8, MA5 = 5:8, MA7.5 = 5[♂]:7; 5-HT: SAL = 8:8, MA5 = 8:8, MA7.5 =

8:7^β; NE: SAL = 7^β:8, MA5 = 8:8, MA7.5 = 7:7^β; P20 DA: SAL = 5^β:8, MA5 = 7^β:8, MA7.5 = 6^β:5^β; 5-HT: SAL = 8:9, MA5 = 9:9, MA7.5 = 7^β:9; NE: SAL = 8:9, MA5 = 9:9^β, MA7.5 = 8:9^β. ^βOne outlier was excluded because levels were >3 standard deviations from the group mean. *Inset* represents significant main effect. Data are LS means ± SEM; **p* < .05

Fig. 4.

Prefrontal cortex monoamine levels [pg/mg tissue, for dopamine (DA; **a, d**); serotonin (5-HT; **b, e**); and norepinephrine (NE; **c, f**)] as a function of treatment and rearing on P15 and P20. P15 DA: **a** No main effects or interactions were observed for DA. P15 5-HT: **b** A main effect of treatment ($F_{2,26} = 19.57$, $p < 0.0001$) showed that MA animals, regardless of dose (MA5: 73.6 ± 15.9 pg/mg and MA7.5: 60.1 ± 18.0 pg/mg) had significantly lower 5-HT levels compared with SAL animals (175.1 ± 15.9 pg/mg, p 's < 0.0001). No interaction was observed. P15 NE: **c** There was a main effect of treatment ($F_{2,23} = 112.17$, $p < 0.0001$), in which MA5 and MA7.5 animals (22.3 ± 5.0 pg/mg and 24.7 ± 5.5 pg/mg, respectively) had significantly lower NE levels compared with SAL (112.1 ± 5.3 pg/mg, p 's < 0.0001). P20 DA: **d** There was no treatment or rearing main effect; however, a significant treatment $\times$ rearing interaction ($F_{2,18} = 3.76$, $p < 0.05$) showed that MA7.5/standard animals had higher DA levels compared with MA7.5/barren animals. Additionally, in standard animals, the MA7.5 animals had higher DA levels compared with SAL ($p < 0.04$) and MA5 animals ($p < 0.01$). P20 5-HT: **e** A main effect of treatment ($F_{2,26} = 95.7$, $p < 0.0001$) revealed that MA5 and MA7.5 animals (53.7 ± 12.9 pg/mg and 42.7 ± 13.2 pg/mg, respectively) had significantly lower 5-HT levels compared with SAL animals (220.3 ± 12.9 pg/mg, p 's < 0.0001). There was also a main effect of rearing ($F_{1,14} = 4.65$, $p < 0.05$; *inset*). Barren animals had increased 5-HT levels compared with standard animals. The interaction was not significant. P20 NE: **f** A main effect of treatment ($F_{2,29} = 29.5$, $p < 0.0001$) revealed that MA5 and MA7.5 animals (36.3 ± 14.2 pg/mg and 23.5 ± 16.4 pg/mg, respectively) had significantly lower NE levels compared with SAL animals (168.0 ± 15.2 pg/mg, p 's < 0.0001). No other effects were found. Group sizes from *left to right*, P15 DA: SAL = 6:5, MA5 = 4:5 β , MA7.5 = 4:3 β ; 5-HT: SAL = 8:8, MA5 = 8 β :8, MA7.5 = 8:5 β ; NE: SAL = 6 β :7, MA5 = 8:7 β , MA7.5 = 8:5 β ; P20 DA: SAL = 4:6, MA5 = 4 β :4 β , MA7.5 = 2 β :4 β ; 5-HT: SAL = 7 β :9, MA5 = 7 β :8 β , MA7.5 = 8 β :8; NE: SAL = 7 β :8 β , MA5 = 8:9 β , MA7.5 = 6 β :

7^β. ^βOutlier excluded because levels were being >3 standard deviations from the group mean. *Inset* represent significant main effects. Data are LS means ± SEM; * $p < .05$

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Table 1

Offspring mortality

	P15		P20	
	Standard	Barren	Standard	Barren
SAL	0/8 (0 %)	0/8 (0 %)	0/8 (0 %)	0/9 (0 %)
MA 5	0/24 (0 %)	7/28 ^{**} β (25 %)	1/24 (4 %)	8/30 [*] β (27 %)
MA 7.5	1/24 (4 %)	7/29 [*] β (24 %)	4/24 (17 %)	9/30 β (30 %)

^{*}
 $p < 0.05$;

^{**}
 $p < 0.01$ compared with standard within treatment (SAL, MA 5, MA 7.5);

β
 $p < 0.05$ (1-tailed) compared with SAL within rearing (standard, barren)

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Table 2

Organ weights, percentage of body weight

P15											
Standard						Barren					
SAL	MA5	MA7.5	SAL	MA5	MA7.5	SAL	MA5	MA7.5	SAL	MA5	MA7.5
Adrenals	0.030 ± 0.002	0.026 ± 0.002	0.033 ± 0.002	0.032 ± 0.002	0.033 ± 0.002	0.033 ± 0.002 [#]	0.026 ± 0.002	0.034 ± 0.003	0.033 ± 0.003	0.035 ± 0.003	0.036 ± 0.003
Spleen	0.507 ± 0.033	0.413 ± 0.033 [#]	0.349 ± 0.033 [#]	0.452 ± 0.035	0.305 ± 0.033	0.316 ± 0.035	0.316 ± 0.035	0.321 ± 3.479	.0469 ± 3.500	0.296 ± 3.131	9.098 ± 3.503
Thymus	0.335 ± 0.018	0.350 ± 0.019	0.328 ± 0.019	0.300 ± 0.020	0.21 [*] ± 0.019 ^{**}	0.198 ± 0.020 ^{**}	0.198 ± 0.020 ^{**}	0.332 ± 0.038	0.329 ± 0.370	0.247 ± 0.035	0.260 ± 0.037

Within age effects:

^{*} *p* < 0.05 compared with SAL, within rearing (standard, barren);

[#] *p* < 0.05 compared with standard within treatment (SAL, MA5, MA7.5)

Table 3

Offspring metabolite content (pg/mg)

P15													
Standard							Barren						
SAL							SAL						
MA5							MA5						
MA7.5							MA7.5						
SAL							SAL						
MA5							MA5						
MA7.5							MA7.5						
SAL							SAL						
MA5							MA5						
MA7.5							MA7.5						
SAL							SAL						
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MA7.5							MA7.5						
SAL							SAL						
MA5							MA5						

STR neostriatum, HIP hippocampus, PFC prefrontal cortex

β One outlier excluded due to metabolite content ±3 standard deviations from the group mean

Within age effects:

* *p* < 0.05 compared with SAL, within rearing (standard, barren);

[#] *p* < 0.05 compared with standard within treatment (SAL, MA5, MA7.5)

P15 STR For DOPAC, a main effect of treatment (*F*_{2,26} = 10.23, *p* < 0.0006) showed that MA5 and MA7.5 animals (467.9 ± 92.6 and 412.0 ± 99.2 pg/mg, respectively; no difference between the two), regardless of housing, had significantly lower levels compared with SAL animals (912.1 ± 92.6 pg/mg (*p*'s < 0.003). There was also a main effect of rearing (*F*_{1,13} = 15.78, *p* < 0.002); in which barren cage animals (848.5 ± 89.4 pg/mg) had increased DOPAC compared with standard cage animals (346.2 ± 89.4 pg/mg; *p* < 0.002). A significant treatment × rearing interaction (*F*_{2,26} = 32.38, *p* < 0.001) showed that SAL/barren animals had greater DOPAC compared with SAL/standard animals (*p* < 0.001) and MA5/barren and MA7.5/barren animals (*p*'s < 0.001). For HVA, there was a main effect of treatment (*F*_{2,27} = 5.55, *p* < 0.01) in which SAL animals had higher HVA levels compared with MA5 animals (1269.8 ± 82.9 pg/mg vs. 971.7 ± 82.9 pg/mg; *p* < 0.008), but not MA7.5 animals (1085.6 ± 88.3 pg/mg). No other effects were noted. 5-HIAA showed a main effect of treatment (*F*_{2,27} = 37.95, *p* < 0.0001), in which levels were significantly decreased in MA5 and MA7.5 animals (453.0 ± 25.7 pg/mg, respectively) but differed from SAL animals (1345.2 ± 63.0 pg/mg). The main effect of rearing (*p* < 0.07) nor the interaction were significant

P20 STR For DOPAC, the main effect of treatment (*F*_{2,30} = 61.08, *p* < 0.0001) showed that MA5 and MA7.5 animals (663.3 ± 55.0 pg/mg and 494.2 ± 61.0 pg/mg, respectively), did not differ from each other, but had lower levels compared with SAL animals (1345.2 ± 63.0 pg/mg). No other effects were found for DOPAC. For HVA, no main effects or interaction was evident. For 5-HIAA, there was a significant main effect of Treatment (*F*_{2,30} = 82.08, *p* < 0.0001), in which MA-dosed groups (*p*'s < 0.0001) had lower 5-HIAA levels compared with SAL animals; MA groups did not differ from each other

P15 HIP Hippocampal analyses showed no main effects or treatment × rearing interaction for DOPAC or HVA. For 5-HIAA the main effect of treatment (*F*_{2,28} = 65.82, *p* < 0.0001) showed that MA5 and MA7.5 animals (167.7 ± 19.8 pg/mg and 151.8 ± 20.6 pg/mg, respectively, with no difference between them) had significantly decreased 5-HIAA levels compared with SAL animals (431.4 ± 19.8 pg/mg, *p*'s < 0.0001). A main effect of rearing was also seen (*F*_{1,14} = 9.42, *p* < 0.009), in which barren animals (288.0 ± 17.6 pg/mg) had increased 5-HIAA levels compared with standard animals (212.6 ± 17.2 pg/mg). No interaction was evident

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*P20 HIP*No main effects or interaction were observed for DOPAC or HVA at P20. For 5-HIAA, there was a main effect of treatment ($F_{2,31} = 133.66, p < 0.0001$); in which MA5 and MA7.5 animals (191.8 ± 18.3 pg/mg and 155.8 ± 18.1 pg/mg, respectively) had significantly less 5-HIAA compared with SAL animals ($504.1 \pm 18.1, p$'s < 0.0001), but the two MA groups did not differ from each other. No rearing or treatment $\times$ rearing interaction was found

*P15 PFC*No main effects or interactions were observed for HVA, however, for HVA, there was a main effect of treatment ($F_{2,22} = 6.65, p < 0.006$); in which MA5 and MA7.5 animals (88.0 ± 11.5 pg/mg and 76.9 ± 12.8 pg/mg, respectively) had significantly lower HVA levels compared with SAL animals (134.0 ± 12.4 pg/mg, p 's < 0.001). For 5-HIAA, there was a significant main effect of treatment ($F_{2,27} = 14.2, p < 0.0001$). Regardless of housing, the MA5 and MA7.5 animals (120.7 ± 20.8 pg/mg and 115.7 ± 23.9 pg/mg, respectively) had significantly lower 5-HIAA levels compared with SAL animals (251.4 ± 20.8 pg/mg, p 's < 0.0003)

*P20 PFC*There were no significant main effects or interactions for DOPAC or HVA at P20. For 5-HIAA, there was a main effect of treatment ($F_{2,29} = 90.5, p < 0.0001$); in which MA5 and MA7.5 animals (115.7 ± 13.2 pg/mg and 98.2 ± 13.4 pg/mg, respectively) had significantly lower 5-HIAA levels compared with SAL animals (262.4 ± 13.5 pg/mg, p 's < 0.0001). A treatment $\times$ rearing interaction was observed ($F_{2,29} = 5.44, p < 0.01$), in which MA7.5/barren animals had higher 5-HIAA levels compared with MA7.5/standard animals, and the difference in SAL- and MA-treated animals was the same as described for the main effect