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Prepubertal environmental enrichment prevents dopamine dysregulation and hippocampal hyperactivity in MAM schizophrenia model rats.

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Abstract

Background—Schizophrenia (SCZ) is a neurodevelopmental disorder with a progressive, prolonged course. Early prevention for SCZ is promising, but overall lacks support from preclinical evidence. Previous studies have tested environmental enrichment (EE) in certain models of SCZ and discovered a broadly beneficial effect in preventing behavioral abnormalities relevant, yet not specific, to the disorder. Nonetheless, whether EE can prevent dopamine (DA) dysregulation, a hallmark of psychosis and SCZ, had not been tested

Methods—Using the methylazoxymethanol acetate (MAM) rat model of schizophrenia and saline-treated controls (SAL) we investigated the long-term electrophysiological effects of prepubertal (postnatal day 21–40) EE on DA neurons, pyramidal neurons in the ventral hippocampus (vHipp), and projection neurons in the basolateral amygdala (BLA). Anxiety-related behaviors in the elevated plus maze and locomotor responses to amphetamine were also analyzed.

Results—Prepubertal EE prevented the increased population activity of DA neurons and the associated increase in locomotor response to amphetamine. Prepubertal EE also prevented the hyperactivity in the vHipp, but did not prevent the hyperactivity in the BLA. Anxiety-like behaviors in MAM rats were not ameliorated by prepubertal exposure to EE.

Conclusions—20-day prepubertal EE is sufficient to prevent DA hyperresponsivity in the MAM model, measured by electrophysiological recordings and locomotor response to stimulant. This effect is potentially mediated by normalizing excessive firing in the vHipp without affecting anxiety-like behaviors and BLA firing. This study identified EE as a useful preventative approach which may protect against the pathophysiological development of SCZ.

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Keywords

schizophrenia; environmental enrichment; dopamine; ventral tegmental area; ventral hippocampus; amygdala

Introduction

Schizophrenia (SCZ) has long been proposed to be a neurodevelopmental disorder (1, 2), and its etiology involves genetic predisposition (3) and environmental factors (4), which collectively interfere with brain development (5–7). As a core feature of SCZ, psychosis typically manifests relatively late in the clinical course, often preceded by an extended prodromal stage in adolescence (8, 9). Several meta-analyses have concluded that the risk of transition to psychosis can be reduced by active intervention during the prodromal phase, including antipsychotic medication, nutritional support, cognitive behavioral therapy, psychoeducation, and exercise (8, 10–12), although not all have proven effective upon replication. In addition to the plausible preventative efficacy against positive symptoms, recent studies also suggest that early intervention may also reduce the risks for the emergence of other symptom domains (13).

Whereas clinical evidence suggests that both early-life pharmacological and environmental interventions are potentially effective in preventing SCZ, the former is relatively disadvantageous due to ethical limitations, economical infeasibility, and potential side effects given that most individuals will never transition to psychosis (13, 14). Early exposure to environmental enrichment (EE) is a form of behavioral intervention that is broadly beneficial to a wide range of neuropsychiatric conditions, including SCZ (15–18). For example, Raine et al. reported that a 2-year environmental intervention at age 3–5 might protect against behavioral manifestation of SCZ in early adulthood (19). In terms of preclinical research, several studies suggested that early EE can prevent selective behavioral abnormalities relevant, but not specific, to SCZ, such as locomotor hyperactivity, social cognition, and sensorimotor gating in SCZ models involving drug treatment (20–22), genetic manipulation (23, 24), and lesion (25).

However, key preclinical evidence supporting the preventative efficacy of EE is still lacking, because beyond its beneficial effects on SCZ-relevant behavioral endpoints, whether EE can prevent other functional deficits of SCZ is largely unknown. A recent study attempted to address this knowledge gap by testing EE in methylazoxymethanol (MAM) GD17 model (26) of SCZ, in which Bator et al. (27) found that 7-day juvenile exposure to EE not only prevented SCZ-related behavioral deficits, but also the decrease in the expression of glutamate decarboxylase 67. Although this recent study extended the current scope of EE research by indicating EE's efficacy against GABAergic pathologies implicated in the pathogenesis of SCZ (28), several key questions remain unanswered. In particular, whether EE can prevent dopamine (DA) dysfunction and hippocampal hyperactivity, core features of the pathophysiology of SCZ (5, 29), is still unknown (30).

Using the MAM model, the main goal of this study is to understand the long-term electrophysiological impacts of prepubertal EE on the regulation of the DA system of adult

animals. Given that hippocampal hyperactivity and the associated DA dysregulation are robust features of SCZ (31), we also examined the impact of EE on neuronal firing in the ventral hippocampus (vHipp), an upstream regulator of the midbrain DA system (32, 33). Given the high comorbidity between anxiety and SCZ (34) and its implication in the pathogenesis and pathophysiology (35–38), we also determined whether prepubertal EE can ameliorate anxiety and the hyperactivity in the basolateral amygdala (BLA) in adult MAM rats (39).

Methods

Animals.

Timed pregnant Sprague–Dawley rats (Envigo) were obtained at gestational day (GD) 14. At GD17, pregnant rats were injected with 0.9% saline or MAM (20mg/kg, i.p.; MRI Global). Animals were housed in a temperature- (22°C) and humidity-controlled (47%) environment (12-hr light/dark cycle; lights on at 7 am) with *ad libitum* access to food and water. To avoid litter effects, individual experimental groups were formed by animals from at least 3 litters (range: 3–6) and counterbalanced across all experiments. Experiments were conducted according to the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experimental design.

At postnatal day (PD) 21, male offspring prenatally exposed to saline (SAL) or MAM were randomly assigned into a regular housing environment (RE) throughout the study or an enriched environment (EE) for 20 days until PD40. EE rats were returned to RE on PD41, and stayed in RE until the end of experiments. At PD65–71, rats were tested for performance in the elevated plus maze (EPM, PD65–66) and amphetamine-induced hyperlocomotion (AIH, PD69–71). Following a week of recovery (>PD77), animals were randomly assigned into *in vivo* single-unit recording experiments (Figure 1). Separate cohorts of rats were also enriched during adulthood (PD65–84), juvenility (PD21–30), and adolescence (PD31–40) to assess possible age-dependence of EE effects. Timeline is shown in Figure 3.

Environmental conditions.

Animals in RE were pair- or triple-housed in typical rodent cages (L38cm×W26cm×H18cm). The EE paradigm was modified from previously established protocols (40, 41). Briefly, rats were group-housed in five large plastic tubs (L93cm×W53.3cm×H49.5cm) containing objects including toys and tunnels of different shapes, running wheels, chewing materials, and plastic ladders attached to a metal platform. The enrichment boxes also contained increased nesting materials and an increased amount of beddings, which were changed twice a week. The orientation and the color of the objects were altered three times a week.

Behavioral Experiments.

Experiments were conducted during the dark cycle (7:00pm – 7:00am).

Elevated plus maze (EPM).—Animals were first habituated to the testing room for 90 minutes. Rats were introduced to the central area facing an open arm, and their movements were recorded for 5 min. Time spent in open arms and the number of open arm entries were measured to index anxiety-like behaviors.

Amphetamine-induced hyperlocomotion.—Locomotor activity was assessed in open-field chambers (Coulbourn Instruments) with ambulatory movement in the x-y plane recorded for 30 minutes. Rats were next injected with d-amphetamine sulfate (0.75 mg/kg, i.p.; Sigma), followed by the recording of locomotion for 60 minutes. Data were computed in 5-min bins for time-course analysis, and the total distance traveled post-amphetamine was calculated.

In vivo electrophysiology.

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.; Sigma) and mounted on a stereotaxic frame (Kopf; Tujunga, CA). See Supplement for details.

Glass electrodes were lowered through six to nine vertical tracks in a predetermined pattern within VTA (A/P: −5.4mm from bregma, M/L: ±0.6mm, and D/V: 6.5–9.0mm from brain surface (42, 43)). Spontaneously active DA neurons were identified based on well-established criteria (Figure S1A–B) (44).

Spontaneously active neurons were recorded in the vHipp by four to six tracks (A/P: −5.5 to −5.9mm, M/L: ±4.6–4.8mm from Bregma, D/V: −5.5 to −8.5mm from brain surface). Putative pyramidal neurons were identified based on published criteria of firing rate <2 Hz (45–47), which was also validated in this study. Fast-spiking vHipp interneurons were defined by average firing rate >4 Hz (47) and spike duration <1.0ms, from peak to valley. For BLA recording, single-unit activities were recorded by four to six vertical tracks (A/P: −3.0 to −3.4 mm, M/L: ±4.6–4.8 mm from Bregma, D/V: −6.0mm to −8.5 mm from brain surface). Putative projection neurons were identified based on previously published criteria (see Supplement).

Statistical analysis.

All results are presented as mean ± SEM, and statistical calculations were performed using GraphPad Prism 8. Data were tested for normal distribution (Kolmogorov-Smirnov normality test) and subsequently analyzed with two-way ANOVA with treatment (SAL or MAM) and environment (RE or EE) as main factors, or two-way repeated measure ANOVA (in AIH experiment) with the developmental condition (SAL:RE, SAL:EE, MAM:RE, or MAM:EE) as a main factor and time/bin as a repeated measure factor. Tukey's *post hoc* test was used when a significant main effect or interaction was detected. Differences were considered significant at $p<0.05$.

Results:

Prepubertal EE prevented the electrophysiological and behavioral phenotypes of DA dysregulation in adult MAM rats.

Consistent with previous reports (48, 49), SAL:RE (n=8, 62 neurons) rats displayed an average of 1.0±0.1 spontaneously active DA neurons per electrode track (Figure 2A). A two-way ANOVA revealed a significant main effect of EE [$F_{environment}(1,29)=12.91$, $p<0.01$] and a significant interaction between treatment and environment [$F_{interaction}(1,29)=6.913$, $p<0.05$]. Tukey's *post hoc* tests revealed that MAM:RE group (n=9, 94 neurons) showed greater population activity (1.4±0.1 cells/track, $p<0.05$). Compared to MAM:RE group, MAM:EE group (n=8 rats, 47 neurons) showed significantly lower population activity (0.8±0.1 cells/track; $p<0.001$). Furthermore, population activity in MAM:EE *vs* SAL:RE groups were not significantly different ($p>0.05$). Prepubertal EE did not have significant effects in SAL animals, as SAL:EE group (n=8, 52 neurons) showed an average of 0.9±0.1 cells/track, which was not significantly different from SAL:RE group ($p>0.05$). SAL:RE rats displayed an average firing rate of 3.5±0.3 Hz, with 26.7±3.5% spikes fired in bursts, consistent with previous reports (48, 49). The firing rate (3.5±0.3, 3.4±0.2, and 3.1±0.3 Hz) and %SIB (21.1±3.0, 26.1±2.6, and 20.2±3.7%, in SAL:EE, MAM:RE, and MAM-EE groups, respectively) did not differ significantly (Figure S1C–D, $p>0.05$; two-way ANOVA). Further analysis of population activity throughout the mediolateral VTA divisions revealed that the increased DA population activity in MAM:RE rats was confined to the medial and the lateral VTA (Figure 2B), consistent with our previous report (50). We found the EE was effective in reducing MAM-induced DA hyperactivity across all subregions (MAM:EE vs. MAM:RE, $p<0.01$ in medial VTA; $p<0.05$ in central and lateral VTA; *Tukey's post hoc* tests following two-way ANOVA).

Locomotor response to amphetamine is heightened in adult MAM rats (48, 49, 51, 52). Consistently, MAM:RE (n=12) group showed significantly higher levels of locomotor activity in response to amphetamine administration (0.75 mg/kg; i.p.) compared to SAL:RE group (n=13) [Figure 2C; $p<0.01$ at 40- and 45-min, $p<0.05$ at 60-min; Tukey's *post hoc* test following repeated measures two-way ANOVA on post-amphetamine movement; interaction of developmental condition*time: $F(1,44)=17.46$, $p<0.001$]. In contrast, MAM:EE rats (n=13) showed a significantly lower level of amphetamine-stimulated locomotion compared with MAM:RE rats ($p<0.001$ at 35–50 and 65–70 min; $p<0.01$ at 55–60 min), and were not significantly different from SAL-RE rats ($p>0.05$ at all post-amphetamine timepoints). Furthermore, prepubertal EE in SAL rats did not affect amphetamine-stimulated locomotion (SAL:RE vs SAL:EE rats, n=8, $p>0.05$ at all post-amphetamine timepoints). The total movement post-amphetamine administration revealed a similar result (Figure 2D), as two-way ANOVA indicated a significant interaction between prenatal MAM treatment and prepubertal environment [$F_{interaction}(1,44)=17.46$, $p<0.001$]. MAM:RE rats showed a significantly higher total movement compared with SAL:RE ($p<0.01$) and MAM:EE rats ($p<0.001$).

To examine the age-dependence of EE, separate sets of MAM rats were enriched during PD21–30 or PD31–40 (Figure 3A). Similar to the 20-day EE paradigm, 10-day EE

paradigms were found to be effective in normalizing the electrophysiological phenotypes of DA hyperresponsivity (Figure 3A), although no effect of 10-day prepubertal EE paradigms was observed in AIH or EPM (Figure S2B–C). In contrast, in adult rats the 20-day enrichment paradigm (Figure 3B) did not impact DA population activity. Based on these results, subsequent experiments on BLA and vHipp activities were only conducted with animals exposed to 20-day prepubertal EE.

Prepubertal EE prevented vHipp hyperactivity in adult MAM rats.

An increase in vHipp activity is proposed to underlie the DA system hyperresponsivity in SCZ and animal models (53). We therefore performed recordings from identified pyramidal and putative fast-spiking interneurons in the vHipp (Figure 4A). In total, 50 neurons/5 rats in the vHipp of SAL:RE group were recorded (Figure 4B), which were divided into two populations each with a normal distribution (Kolmogorov-Smirnov test of normality, $p>0.1$) based on previously reported firing rate cut-off of 2 Hz (45). A significant difference in the mean firing rate in the pyramidal vs. non-pyramidal neurons was detected (Figure 3B; $t=12.26$, Welch's correction, $df=23.61$, $p<0.001$), confirming the applicability of this identification criterion.

No changes in the number of spontaneously active pyramidal neurons detected per track across groups were observed (Figure 4C). Consistent with previous data (45, 49), vHipp pyramidal neurons in SAL:RE (28 cells/5 rats) group displayed a mean firing rate of 0.62±0.08 Hz (Figure 4D). A two-way ANOVA revealed a significant effect of MAM [$F_{treatment}(1,125)=8.675$, $p<0.01$], enrichment [$F_{environment}(1,125)=9.717$, $p<0.01$], and their interaction [$F_{interaction}(1,125)=6.396$, $p<0.05$]. Tukey's *post hoc* tests revealed that MAM:RE group (28 cells/6 rats) displayed significantly higher firing rates (1.13±0.11 Hz; $p<0.01$, vs. SAL:RE), which were prevented by prepubertal exposure to EE (MAM:EE, 48 cells/8 rats; 0.60±0.07 Hz; $p<0.001$, vs. MAM:EE). Furthermore, in SAL rats, we did not observe any change associated with EE (SAL:EE, 25 cells/4 rats; $p>0.05$, vs. SAL:RE).

Of the identified non-pyramidal cells (i.e. firing rate >2 Hz), we further separated neurons at relatively higher firing rate (>4 Hz) and with shorter spike duration (<1.0ms, from peak to valley) (Figure 4E). These operationally defined non-pyramidal neurons putatively represent fast-spiking neurons (47, 54). A two-way ANOVA revealed a significant effect of enrichment [$F_{environment}(1,54)=5.50$, $p<0.05$]. Tukey's *post hoc* tests revealed that MAM:EE group (19 cells/7 rats) displayed a significantly higher firing rate (7.97±0.68 Hz), compared to MAM:RE (13 cells/5 rats; 5.93±0.50 Hz; $p<0.05$), SAL:RE (15 cells/4 rats; 5.60±0.32 Hz; $p<0.01$), and SAL:EE (11 cells/3 rats; 5.72±0.32 Hz; $p<0.05$) groups.

Prepubertal EE did not prevent anxiety-like behaviors in EPM and BLA hyperactivity in adult MAM rats.

Heightened anxiety and stress sensitivity are proposed to contribute to the onset of DA dysregulation in MAM rats (39, 55). Confirming previous findings (39, 48), MAM:RE (n=9) rats display higher level of anxiety-like behaviors in EPM, indexed by decreased time spent in open arms [Figure 5A; $F_{treatment}(1,42)=12.84$, $p<0.001$; two-way ANOVA] and decreased percent entries into open arms [Figure 5B; $F_{treatment}(1,42)=11.85$, $p<0.01$; two-way

ANOVA]. *Post hoc* Tukey's tests revealed that MAM:RE rats spent less time in open arms ($p<0.05$) and had lower percent entries into open arms ($p<0.05$), compared to SAL:RE (n=11) rats. Prepubertal EE did not rescue anxiety-like behaviors in MAM rats [open arm time: Figure 5A; $F_{environment}$ (1,42) = 5.251, $p< 0.05$; Tukey's test, MAM:RE vs. MAM:EE (n=16), p=0.577 or percent open arm entries [Figure 5B; $F_{environment}(1,42)=0.1856$, p=0.669]. Overall locomotion, indexed by total arm entries, was not different among groups [Figure 5C; $F_{interaction}(1, 42)=0.3923$, p=0.53]. To assess the effect of EE on anxiety-like behaviors during prepuberty, in a separate group of rats we measured the EPM responses at PD43–44 (Figure S3A). While we observed a main effect of MAM in both open arm time [Figure S3B, $F_{treatment}(1,16)=5.576$, $p<0.05$] and percent open arm entries [Figure S3C, $F_{treatment}(1,16)=5.745$, $p<0.05$], these measures were not different between MAM:RE vs. MAM:EE group in *post hoc* tests, suggesting EE's inability to rescue MAM-induced anxiety starts early in development.

We recorded from putative projection neurons in the BLA, a key region involved in the regulation of fear and anxiety-like behaviors (56). Projection neurons were identified based on previously published criteria (39, 57): (1) bi- or triphasic waveforms, (2) long spike duration (>2 ms), and (3) slow firing rate (<1 Hz) (Figure 5D). No changes in the number of spontaneously active neurons in each track were observed across groups (Figure 5E). For firing rate (Figure 5F), confirming previous results (57), the average firing rate of the recorded putative projection neurons was very low (SAL:RE, n=45 cells/6 rats, 0.21±0.03 Hz). A two-way ANOVA revealed a significant main effect of MAM [$F_{treatment}(1,124)=6.220$, $p<0.05$], but only a trend toward significance for enrichment [$F_{environment}(1,124)=3.504$, p=0.0636]. Tukey's *post hoc* analysis revealed that, compared to SAL:RE and SAL:EE (20 cells/4 rats, 0.1849±0.03 Hz) groups, MAM:RE group (36 cells/5 rats) displayed significantly higher BLA projection neuron firing (0.34±0.04 Hz, $p<0.05$), which was not significantly different from that of MAM:EE rats (28 cells/5 rats, 0.23±0.03 Hz, p=0.19), consistent with the behavioral measures in the EPM. Altogether these data suggest prepubertal EE is not effective in preventing anxiety in terms of EPM or BLA activity in adult MAM rats.

Discussion:

In this study, we examined prepubertal EE for the prevention of psychosis-related dopamine dysregulation in an animal model. Rearing MAM offspring in an enriched environment prepubertally prevented the emergence of DA system hyperresponsivity and vHipp hyperactivity, without affecting anxiety-like responses and the firing of BLA projection neurons. Altogether, these results indicate that early EE might be effective in reducing psychosis-related electrophysiological changes predisposed by early-life risk factors.

We used the MAM GD17 model (26), in which a single injection of a neurospecific, short-acting mitotoxin (58) during late gestation selectively disrupts neurodevelopment (51, 59), resulting in phenotypes in the offspring consistent with SCZ (53, 60). This model is effective in screening preventative strategies due to its ability to recapitulate certain prodromal aspects of SCZ, specifically increased stress susceptibility, anxiety-like response, and hippocampal

pathology during prepuberty (39, 48, 61, 62), before the manifestation of DA-related behavioral abnormalities in adults (51).

Previous research on EE effects in SCZ-relevant models tends to focus on behavioral phenotypes, including sensorimotor gating deficits, social interaction impairment, hyperactivity, and memory deficits (27, 63–65). To our knowledge, the present study represents the first examination of whether EE can protect against psychosis-related dopamine dysregulation, and hence provides novel insight for early intervention. We showed that prepubertal EE is sufficient to prevent the vHipp-driven DA hyperactivity across VTA subregions of adult MAM rats. Noteworthy, the ability of prepubertal EE to prevent DA hyperresponsivity in the lateral VTA (Figure 2B) is strongly consistent with the DA dysfunction in human SCZ, which is most prominent in this region and its target (i.e. associative striatum) (66).

DA hyperresponsivity in SCZ likely originates in its afferent regulators (67), such as the limbic hippocampus which can potently regulate DA system responsivity via a polysynaptic, disinhibitory circuit (33, 68). Thus, aberrant activity in vHipp will induce DA hyperresponsivity through ventral pallidum mediated disinhibition (49). Consistent with this model, anterior hippocampal hyperactivity is reported in SCZ patients (69, 70), which may in turn drive the DA dysfunction (29) that underlies psychosis (71). Moreover, studies of at-risk groups posited a pathogenic role for hippocampal dysregulation in psychotic conversion (72, 73). Importantly, hippocampal parvalbumin (PV) interneuron deficits have been implicated in the pathophysiology of SCZ (74–77). Closely recapitulating this clinical feature, MAM rats display similar abnormalities in the vHipp (a functional equivalent of anterior hippocampus in primates (78, 79)), which leads to VTA DA hyperactivity (49, 51, 80). Altogether, these findings suggest that the hyperdopaminergic state in SCZ might be a consequence of excessive hippocampal activity, driven by the loss of PV or PV interneurons (28, 55).

We found prepubertal EE prevented pyramidal neuron hyperactivity in the vHipp of MAM rats (Figure 4), as well as the DA hyper-responsivity in the VTA (Figure 2). These results are consistent with previous reports using pharmacological (49), genetic (81), and stem-cell-based (45) approaches to modulate vHipp activity and hence to reduce DA hyperactivity. Previous research also indicated that EE attenuates the decrease of the PV interneurons in the hippocampus of MAM rats (82), which could be the structural basis underlying the observed functional prevention. During prepuberty, vHipp interneurons undergo significant development and maturation, characterized by an increase in PV expression and enhanced wrapping of the perineuronal nets (PNNs) (42, 83). PNNs have diverse functions (84), and the protection of PV neurons against cellular damage and oxidative stress is likely relevant to this study. Post-mortem studies have linked PNN loss to the pathogenesis of SCZ (85). Given the preclinical evidence that early EE can increase PNN expression (86, 87), the observed prevention of DA dysregulation might be mediated by positive modulation of PNNs in the vHipp, thus enhancing the resilience of the PV interneurons to increased oxidative stress known to be present in MAM rats (88). Protection of PV interneurons in the MAM vHipp would enhance local inhibition of pyramidal neurons, consistent with the present data in vHipp recording (Figure 4D, E). In contrast, EE in adulthood (PD65–84) was

unable to modulate abnormal DA activity in MAM rats (Figure 3B). Since the PV loss in MAM rats emerges early (62), this failure of adult EE to prevent DA dysregulation suggests that the enrichment paradigm is indeed a preventive rather than therapeutic approach, addressing a need for future research to focus on the translation of EE by identifying the optimal window to apply this early intervention (8, 13). Furthermore, given the important sex differences in SCZ (89) and the sexually dimorphic behavioral effects of EE (90, 91), it is likely that female MAM rats will show significant developmental stage-specific changes that differ from males particularly with respect to the impact of stress (92). Further studies are required to investigate if the findings described here in males would be observed in females as well.

During prepuberty, MAM rats display increased stress susceptibility, heightened anxiety-like responses, decreased adaptability to stress, and increased BLA firing (48, 61). Furthermore, treating anxiety in MAM rats with diazepam during prepuberty prevents the adult DA dysregulation (48). These findings and the epidemiologic evidence on the pathogenic role of stress (93, 94) suggest that abnormal stress vulnerability during critical developmental stages, such as adolescence (95, 96), may lead to later-onset of SCZ-related pathophysiology (55). Previous studies suggest that EE can mitigate the negative behavioral effects of stress by enhancing resilience and stress adaptability (97–99). Furthermore, previous studies have also found that EE can mitigate anxiety and the associated maladaptive structural and molecular plasticity in the BLA, examined shortly after EE (100, 101). However, whether EE can chronically modulate BLA activity is unknown. Intriguingly, PD21–40 EE in MAM rats was not sufficient to prevent anxiety-like responses in the EPM and hyperactivity in identified BLA projection neurons (Figure 5A–C and E). These data point to dissociable behavioral and neurophysiological benefits of early EE in the MAM model. Speculatively, these observed negative results in EPM and BLA-related measurements could be related to the anxiogenic effect of enrichment loss (i.e. returning to RE on PD41), which would be consistent with a previous study reporting a rapid loss of the effect of EE on anxiety in open field test when animals return to home cages (102). This explanation would also be supported by the EPM data measured at PD43–44 (Figure S3), during which we found that EE did not rescue anxiety-like behavior even shortly (i.e. 3–4 days) after the termination of the paradigm. Alternatively, these data also suggest possible sensitive periods for EE to produce long-lasting behavioral and/or neurophysiological benefits, which may partially explain the differential effects of 10-day EE starting PD21 or 31 (Figure 3A and S2). A recent study reported that pre-weaning (PD2–21) enrichment can positively modulate anxiety-related behaviors, spine density, and brain-derived neurotrophic factor in the BLA (103). Thus, the full beneficial effects of developmental EE on adult MAM-related phenotypes is possibly achieved via a sensitive-period-like, sequential mechanism, with prepubertal enrichment selectively targeting vHipp-related pathophysiology and DA dysfunction.

Although many effective enrichment paradigms incorporate an early post-weaning phase, the exact outcomes can vary dramatically depending on EE procedures, with onset, duration, and continuity of EE being critical variables (90).

The generalization of rodent enrichment protocols to humans is challenging, as modifications of rodent housing are not directly comparable to treatments in humans (104). Thus, one should carefully consider control groups when interpreting the EE effects in animals. The RE cages here are similar to a typical rodent housing environment, characterized by reduced and unvarying environmental stimuli. This could be construed as relatively impoverished mainly for a lack of cognitive stimulation, an element provided by most EE cages (90). One interpretation of the present study could be that the observed EE effects against SCZ-relevant changes might be generalizable selectively to a population raised in an impoverished environment, such as individuals with low socioeconomic status (SES), a known risk factor for SCZ (105, 106). Lack of cognitive stimulation is a critical mediator of the low SES effects, which can act synergistically with other prenatal factors to negatively affect neurodevelopment (107). According, while the effect of MAM has been largely attributed to the *in utero* neurodevelopmental disruption (i.e. the "MAM-phenotypes" (26)), current data and the emerging evidence (55) raised the possibility that the adult SCZ-relevant phenotypes of MAM rats might also arise from the impoverished postnatal environment. Whether early EE can specifically counteract the influence of direct neurodevelopmental disruption and/or the impoverished environment in MAM rats warrants future studies.

In summary, the present study supports prepubertal environmental enrichment as a useful preventative approach against the pathophysiological development of SCZ. Although prepubertal EE did not fully prevent abnormal pathophysiology in the adult (such as anxiety-like response and BLA hyperactivity), our results indicate that prepubertal EE is sufficient to prevent DA dysregulation and vHipp hyperactivity in the MAM model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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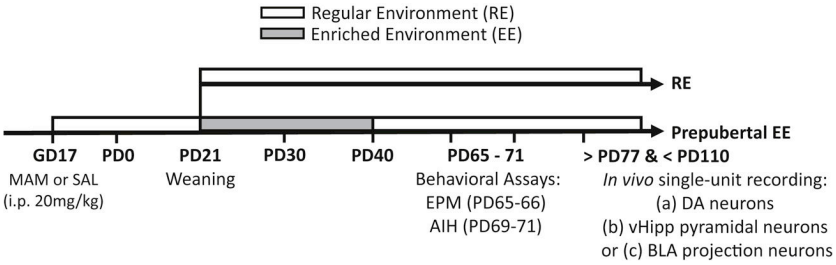

Figure 1. Schematic depiction of the postnatal environmental enrichment protocol for rats treated prenatally with either SAL or MAM at gestational day 17 (GD17).

Animals were weaned at PD21 followed by rearing in RE or EE for 20-days. Behavioral assays were performed in PD65–71, and rats were then randomly assigned into three recording experiments.

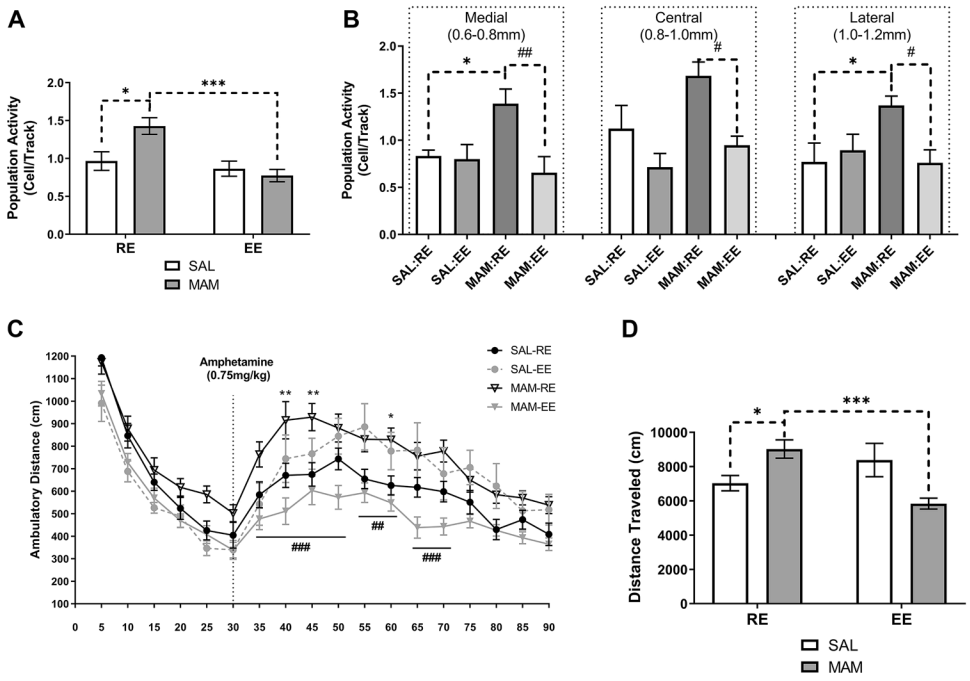

Figure 2. Prepubertal environmental enrichment prevented dopamine hyperactivity in adult MAM rats.

(A) MAM:RE rats displayed an increased number of spontaneously active VTA DA neurons comparing to SAL:RE rats, and prepubertal (PD21–40) exposure to EE prevented the heightened population activity DA neurons (n=8–9). (B) In MAM:RE, the increased in the number of spontaneously active DA neurons was confined to medial and lateral VTA, which were normalized by exposure to EE. (C) The behavioral manifestation of DA hyperresponsivity was measured by AIH, and MAM:RE rats had an augmented locomotor response to amphetamine (0.75 mg/kg; i.p. injection indicated by the dashed line), resulting in (D) increased total distance traveled after amphetamine injection. These changes were prevented by prepubertal EE. (n=8–14). Data are presented as mean ± SEM. *p or #p<0.05; **p or ##p<0.01; ***p or ###p<0.001; * indicated MAM:RE vs. SAL:RE; # indicates MAM:RE vs. MAM:EE

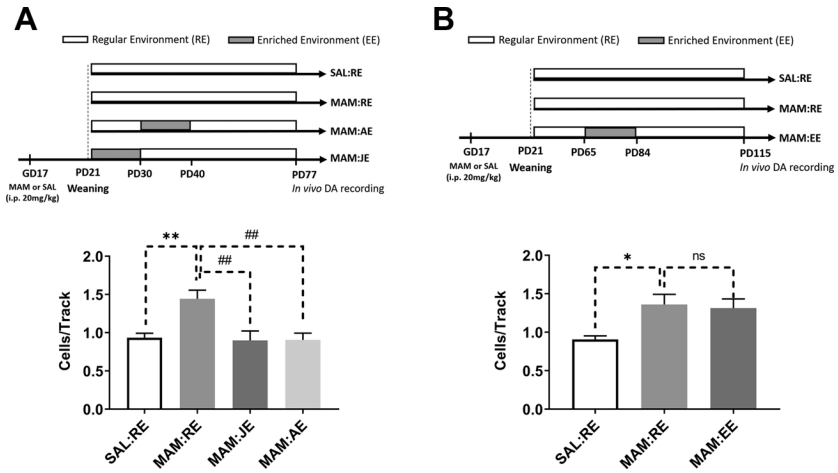

Figure 3. The effects of 10-day prepubertal and 20-day adult environmental enrichment on DA hyperactivity in MAM rats.

(A, top) A separate cohort of rats (n=10) was exposed to early EE during juvenility (JE, PD21–30) and adolescence (AE, PD31–40). (A, bottom) VTA DA neurons population activity was significantly affected by developmental conditions (one-way ANOVA, $F(3,36)=7.110$, $p<0.001$), such that DA hyperactivity induced by prenatal MAM treatment (Tukey's *post hoc* test, MAM:RE vs. SAL:RE, $p<0.01$) was prevented by both JE ($p<0.01$) and AE ($p<0.01$). (B, top) To validate the age-dependent effect of EE, a separate cohort of rats (n=4–6) was enriched during adulthood (PD65–84). (B, bottom) A main effect was detected for developmental conditions [$F(2,12)=5.203$, $p<0.05$], and MAM:RE rats displayed increased population activity (Tukey's *post hoc* test, $p<0.05$), which was not prevented by adult EE ($p>0.05$, MAM:RE vs. MAM:EE). Data are presented as mean ± SEM. *$p<0.05$; **p or ##$p<0.01$; ns: not significant ($p>0.05$). * indicated MAM:RE vs. SAL:RE; # indicates effects of enrichment groups.

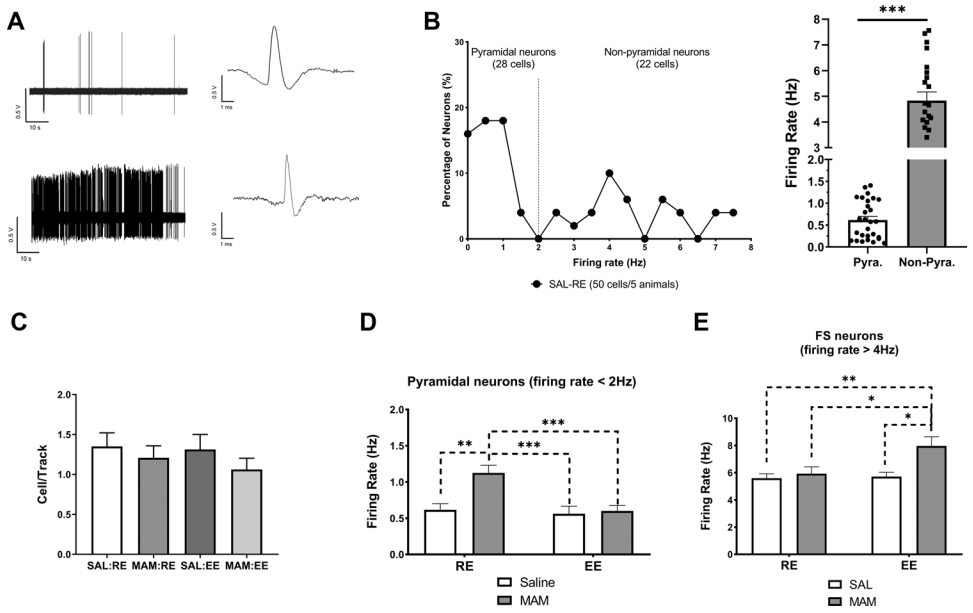

Figure 4. Prepubertal environmental enrichment prevented hyperactivity in vHipp pyramidal neurons.

(A) 1-min segments of spontaneous activity and representative waveform of a putative pyramidal neuron (top) and a putative fast-spiking neuron (bottom) in the vHipp. (B) Neuronal firing rate distribution of neurons detected in SAL:RE rats supports the presence of two populations of vHipp neurons putatively following bimodal distribution that can be separated into two normal distributions (with a 2Hz-cutoff). (C) No change was found in the number of spontaneously active pyramidal neurons per track was detected across groups (n = 4–8 rats). (D) MAM:RE rats displayed increased firing rates of pyramidal neurons in the vHipp, which was prevented by prepubertal EE (25–48 cells/4–8 rats). (E) Of the fast-spiking (firing rate > 4 Hz) non-pyramidal neurons (n=11–19 cells/3–7 rats), a main effect of EE was detected (two-way ANOVA, $F(1,54)=5.500$, $p<0.05$), and MAM:EE rats displayed increased firing rate in fast-spiking cells (Tukey's *post hoc* test). Data are presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. pyra.: putative pyramidal neurons; FS: fast-spiking neurons.

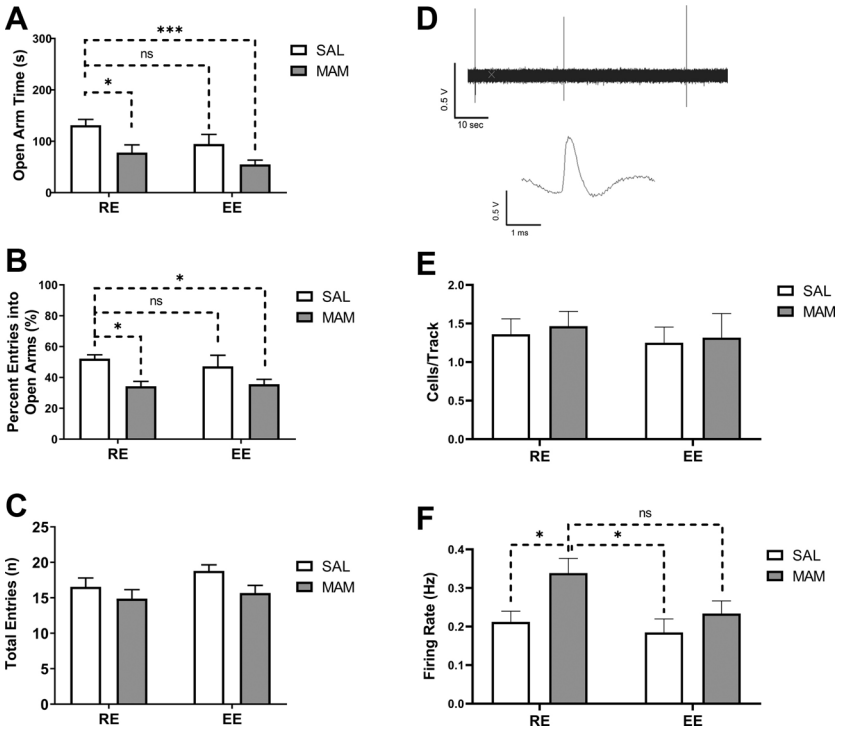

Figure 5. Prepubertal environmental enrichment did not prevent anxiety-like responses in the elevated plus maze or BLA hyperactivity in adult MAM rats.

(A-C) Adult MAM:RE rats, compared to SAL:RE rats, exhibited (A) less time spent in open arms, (B) lower percent of entries into open arms, but (C) no difference in the total number of entries, consistent with increased anxiety. Prepubertal EE did not prevent these changes in MAM rats. (n = 9–16 rats). (D-F) the effect of prepubertal EE on BLA neuronal firing. (D) Representative recording of 1-min spontaneous activity of identified putative BLA projection neurons and its waveform. (E) No change was found in the number of spontaneously active projection neurons detected per electrode track. (F) The hyperactivity of BLA neurons in MAM:RE rats was not prevented by prepubertal exposure to EE (n = 20–45/4–6 rats). Data are presented as mean ± SEM. *p<0.05; ***p<0.001; ns: not significant.

Key Resource Table

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	**Include species and sex when applicable.**	**Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use "this paper" if new.**	**Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.**	**Include any additional information or notes if necessary.**
STERILITE Storage Box		Reference: PMID: 27091967	Mfr. Model # 19453V04	EE box
Combo Chew		Super Pet	MPN:100501560	EE toys
Running Wheel		KAYTEE	MPN:100533403	EE toys
Ladders		Penn Plax	MPN: BA243	EE toys
Play ball pack		Marshall Pet Products	MPN: 572017	EE toys
Lattice Balls with Bells		Penn Plax	MPN: BA-511	EE toys
Color Play Dangly		Oxbow	MPN: 2951	EE toys
Atomic Ball		Ware	MPN: 089348	EE toys
Fintronics Window Discriminator		Fintronics	WDR420	amplifier - for eletrophysiology
Adinstruments Powerlab 8/30 recording unit		Adinstruments		interface - for eletrophysiology
Lab Chart 8		Adinstruments		data acquisition software - for eletrophysiology
GraphPad Prism		GraphPad		data analysis
WPI Glass Capillaries		WPI	1B200F-4	glass eletrode
Med Associates video plus maze - rat		Med associates	ENV-664	for EPM behaviors
True Scan photobeam sensor		Coulbourn Instruments	E63–22	for AIH behaviors