Prepubertal Environmental Enrichment Prevents Dopamine Dysregulation and Hippocampal Hyperactivity in MAM Schizophrenia Model Rats

Supplemental Information

Supplemental Methods

Elevated plus maze (EPM). The EPM apparatus includes two enclosed arms opposed by two open arms (L50cm × W10 cm), forming a cross shape. During the test, the maze was positioned 50 cm above the floor. Each rat was introduced to the central platform facing an open arm, and its movement was recorded for 5 min with a camera overhead. The time spent in arms and entry was determined with reference to two paws and the head. In between sessions, the EPM was cleaned with 75% ethanol and dried with paper towels. To prevent disturbance to the animals in the home cage, when a session is finished, the rat was placed in a separate cage until all the cage mates finished testing. Animals showing low locomotion (defined by fewer than two total entries) or extended freezing on an open arm (defined by freezing behavior lasting more than 30 sec) were excluded (1).

Behavioral analysis. Videos of EPM were manually and independently scored by two to three experimenters blind to the design, and the average value of each animal was used in group analysis.

Single-unit electrodes for *in vivo* electrophysiology. During single-unit recording in anesthetized animals, the body temperature was maintained at 37 °C with a temperature controller (TC-1000, CWE, Inc., PA), and suppression of hind limb withdrawal reflex was monitored periodically by foot pinch. Glass electrode was constructed using an electrode puller (Narishige) with microscopic control and filled with 2 M NaCl containing 2% Chicago Sky Blue (impedance 7 to 15 MΩ in situ).

***In vivo* extracellular recording and analysis.** All the single-unit recordings were acquired with an amplifier (Fintronics, Orange, CT) with the same open filter settings (low pass=50 Hz, high pass=8 kHz,

1000 × gain). The signal was fed to a computer running Lab Chart 8 and displayed on an oscilloscope (B&K Precision, Yorba Linda, CA). Individual neuron data was analyzed by Lab Chart 8 to identify spike duration, and exported to Neuroexplorer (Nex Technologies, Madison, AL) for further analysis of firing rate and burst firing. To reduce animal use, BLA and vHipp recordings were conducted in the same group of animals in the opposite hemispheres at random order.

Identification criteria of VTA DA neurons, vHipp projection neurons, and BLA projection neurons. DA neurons were identified based on well-established criteria (2), specifically (a) action potential duration >2.2 ms, with spike initiation to the maximal negative phase >1.1 ms, (b) biphasic waveform, and (c) irregular firing rate <10Hz. The activity of each identified DA neuron was recorded for 1-3 mins. Three parameters of activity were measured: (a) population activity, i.e., the number of spontaneously active DA neurons detected per electrode track; (b) firing rate; and (c) the percentage of action potentials occurring in bursts. Burst firing of DA neurons was defined as the occurrence of two spikes with an interspike interval of <80 ms, and the termination of the burst was defined as the occurrence of an interspike interval of >160 ms. Putative vHipp pyramidal neurons were identified based on the published criterion of firing rate <2 Hz (3-5). Putative projection neurons were identified based on our previously published criteria: (a) long spike duration (>2.0 msec) (6), (b) slow firing rate (<1Hz) (7, 8), and (c) biphasic waveform (signal to noise ratio >3:1). Once identified, BLA and vHipp activities were recorded for 1.5 – 3 minutes.

Histology. After recordings, rats were euthanized by an overdose of chloral hydrate. The brains were removed, fixed for >24hrs in 8% paraformaldehyde followed by cryoprotection in 25% sucrose (both in 2M phosphate buffers), and sectioned for histological confirmation of the electrode placement based on established protocols (8-10).

Supplemental Figures

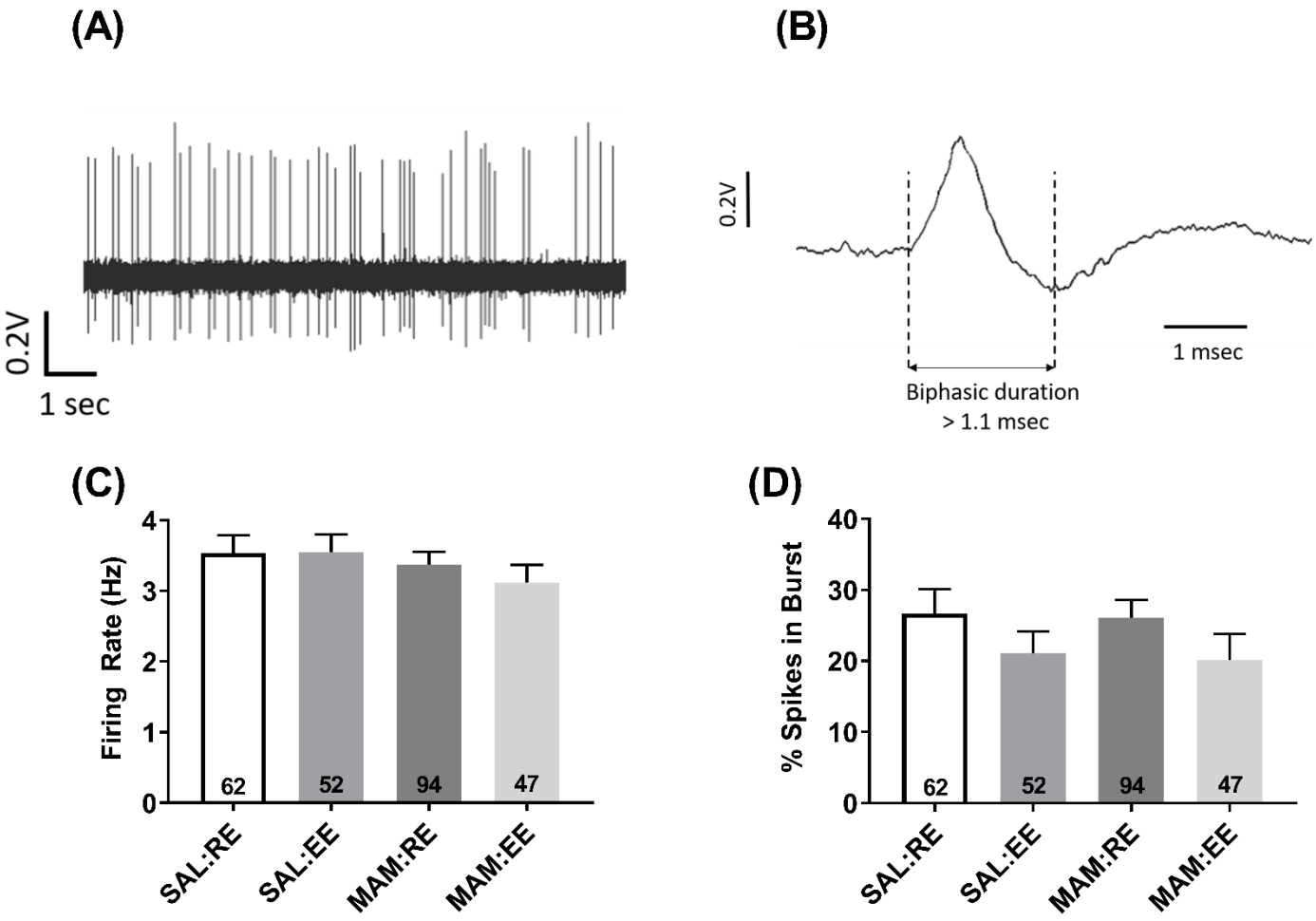

Figure S1. Firing rate and burst firing of the identified DA neurons were not affected by MAM or EE. (A) Representative recording of spontaneous activity of a DA neuron for 10 sec. (B) Representative waveform of a putative DA neuron. (C, D) The firing rate and percentage of the spikes firing in burst were not different among groups (two-way ANOVA, $p>0.05$ for treatment, environment, and their interaction).

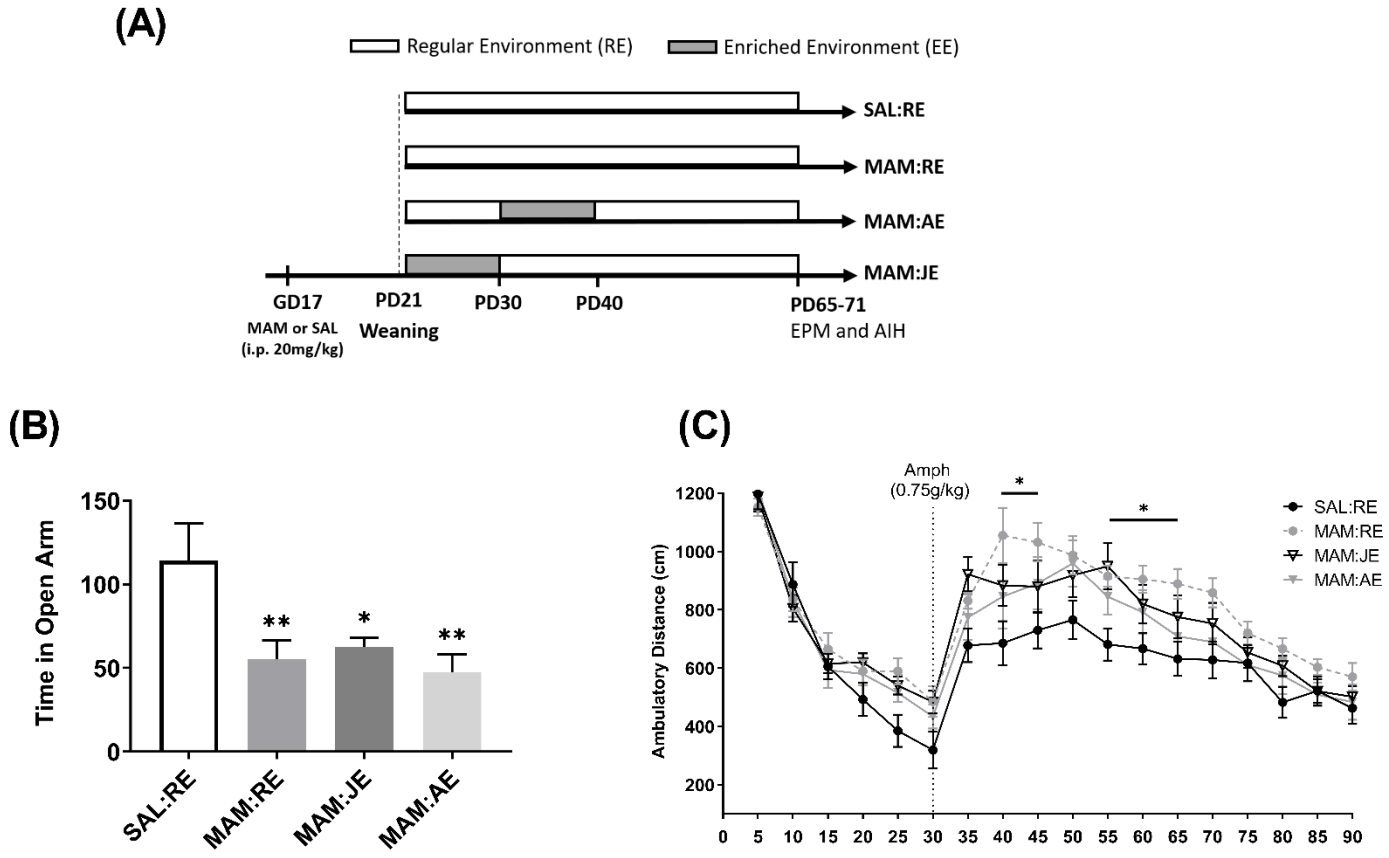

Figure S2. The effect of 10-day EE on adult anxiety and amphetamine-induced locomotion in MAM rats. (A) Overall design of a separate cohort of rats exposed to early EE during juvenility (JE, PD21-30) and adolescence (AE, PD31-40). (B, C) Neither JE nor AE was sufficient to prevent MAM-induced behavioral abnormalities in EPM and AIH. Data are presented as mean ± SEM. **/##p<0.01; */#p<0.05. * vs. SAL:RE; # vs. MAM:RE.

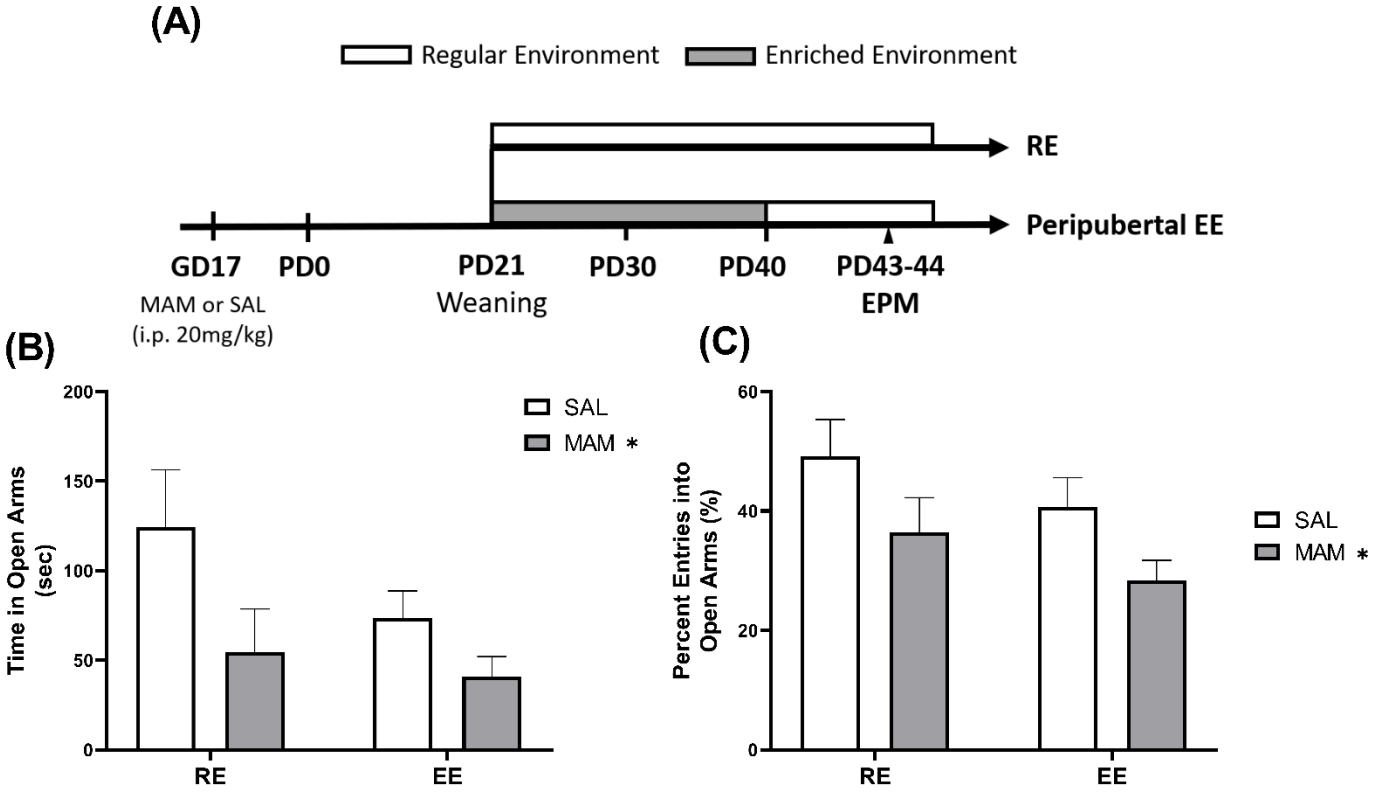

Figure S3. Effects of peripubertal environmental enrichment on adolescent anxiety. (A) A separate cohort of rats was tested for anxiety-like behaviors in the EPM at PD43-44, three days after the end of the enrichment paradigm. (B) MAM treatment significantly affected the time spent in open arm (n=4-6; $F_{treatment}(1,16) = 5.576$, $p<0.05$), and this effect was not reversed by peripubertal EE (Tukey's *post hoc* test, $p>0.05$, MAM:RE vs. MAM:EE). (C) Similarly, in anxiety-like behavior indexed by percent entries into open arms, while a main effect of MAM was detected ($F_{treatment}(1,16) = 5.745$, $p<0.05$), this effect of MAM was not reversed by EE (Tukey's *post hoc* test, $p>0.05$, MAM:RE vs. MAM:EE).

Supplemental References

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