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Brief communication

Perinatal exposure to environmental polychlorinated biphenyls sensitizes hippocampus to excitotoxicity *ex vivo*

Kyung Ho Kim [1], Isaac N. Pessah [*]

Department of Molecular Biosciences, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616, USA

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SUMMARY

Ortho-substituted polychlorinated biphenyls (PCBs) are a concern to human developmental health. Rat dams were exposed to an environmentally relevant mixture of PCBs, Aroclor 1254, or pure congener PCB 95 (6 mg/kg/day) during the perinatal period (GD 5 through PD 21). Hippocampal slices prepared from offspring 1–3 weeks post-weaning were tested for persisting changes in sensitivity to the excitotoxicant picrotoxin. Hippocampal slices were placed on multielectrode arrays. Field excitatory postsynaptic potentials (*f*EPSPs) were recorded from Schaffer Collateral/Commissural fibers in striatum radiatum of the CA1 region in response to single pulse stimuli. After recording baseline excitability, $GABA_A$ receptors were blocked by inclusion of picrotoxin (100 μM) in the aCSF perfusate. Picrotoxin produced negligible changes in *f*EPSP slope in slices isolated from offspring exposed to vehicle, whereas slices from either PCB test group invariably showed >200% ($p < 0.01$) synaptic facilitation. Picrotoxin produced prominent after-discharges (epileptic wave forms) in the evoked potentials measured from PCB exposed, but not control, hippocampal slices. These results show that developmental exposure to non-coplanar PCBs is sufficient to produce changes in synaptic plasticity that can be unmasked in the presence of $GABA_A$ receptor deficits that persist 1–3 weeks after exposure ceased. Developmental exposure to PCBs may sensitize seizure susceptibility postnatally, especially in susceptible populations with $GABA_A$ receptor signaling deficits.

1. Introduction

Recent studies with acutely dissociated rat hippocampal slices showed that *in vitro* exposure to nanomolar concentration of non-coplanar polychlorinated biphenyls (PCBs) of concern to human developmental health can rapidly alter synaptic transmission within CA1 (Kim et al., 2009). Whole-cell voltage clamp recordings obtained from neurons within the primary auditory cortex (A1) *in vivo* indicated that rats exposed during gestation and lactation (perinatally) to a non-coplanar PCB found in environmental samples and human tissues – 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95) – exhibited significant alterations in the balance of excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) (Kenet et al., 2007). These changes were associated with abnormal development of receptive fields within A1 (Kenet et al., 2007). Perinatal exposure to a complex PCB mixture, Aroclor 1254, was shown to alter activity dependent dendritic growth in several brain regions and impaired Morris water maze performance in weanling rats (Yang et al., 2009). These results suggested that non-coplanar PCBs are capable of shifting the balance of excitatory and inhibitory neurotransmission as a consequence of developmental exposure. Such effects could have far reaching ramifications on the normal formation and excitability of neural networks. In the pilot study reported here, rats were exposed during the perinatal period via maternal dosing of PCB 95, Aroclor 1254, or corn oil vehicle. Hippocampal slices were prepared from the offspring, placed on microelectrode arrays (MEAs), and electrophysiological recordings were performed *ex vivo*. Perinatal exposure to either PCB 95 or the complex mixture Aroclor 1254 significantly enhanced picrotoxin-triggered synaptic facilitation and produced prominent after discharges in *f*EPSP waveforms compared to those prepared from vehicle controls. Developmental exposure to non-coplanar PCBs, either singly as a pure congener or as a complex mixture, persistently enhances susceptibility to a $GABA_A$ receptor signaling deficit.

* Corresponding author. Tel.: +1 530 752 6696; fax: +1 530 752 4698.
E-mail address: inpessah@ucdavis.edu (I.N. Pessah).
[1] Current address: Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, 215 Biomedical Science Building, 103 Daehak-Ro, Chongro-Gu, Seoul 110-799, Republic of Korea.

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2. Material and methods

2.1. Animals and developmental exposure to PCBs

Time-mated rat dams (Sprague Dawley; Charles River Laboratories, Hollister, CA) were randomly assigned to a treatment group. Aroclor 1254 (lot number 124-191-B) and 2,2′,3,5′,6-pentachlorobiphenyl (PCB95, >98%) were purchased from AccuStandard (New Haven, CT) and dissolved in 100% corn oil at a final concentration of 24 mg/ml. Dams were offered a cornflake with 0 or 6 mg/kg/dayPCB 95 or Aroclor 1254 (75–100 μl corn oil) at 5 pm from gestational day (GD) 5 to postnatal day (PD) 21. The observer documented complete ingestion of each dose. Animals were kept on a12-h light/dark cycle throughout the study and had access to food and water *ad libitum*. The day of birth was counted as PD 0. Each litter was kept with their biological mother until weaning (PD 21). After weaning, males from each litter were separated by treatment group and housed thereafter 2–3 animals per cage. All procedures conformed to NIH guidelines and were approved by the University of California Davis Institutional Animal Care and Use Committee.

2.2. Hippocampal slice preparation and electrophysiology

The hippocampus was dissected from males (ages 4–6 weeks; PD 28–42) from each treatment group. The method for preparing slices from rat hippocampus and recording field excitatory postsynaptic potential (*f*EPSP) on MEAs was previously described (Kim et al., 2009). Briefly, measurements were made with constant perfusion of artificial cerebral spinal fluid (aCSF; 35 °C) at the slice interface. Single-pulse, biphasic stimuli (10–80 μA, 0.1 ms) were delivered to Schaffer-Collateral/Commissural pathway within the striatum radiatum in CA1 region at 0.05 Hz (i.e., once every 20 s) (Fig. 1A, left panel). The evoked field excitatory postsynaptic potentials (*f*EPSPs) were acquired from recording electrodes chosen based on their proximity to the stimulating electrode. *f*EPSPs were sampled at 20 kHz using a MED 64 multi-channel amplifier, digitized and graphically displayed using Performer® software (Alpha Med Scientific Inc. Osaka, Japan). Baseline *f*EPSPs were experimentally set between 50 and 60% of maximum of amplitude for each slice. During the stabilization period (at least 30 min), slices that exceeded more than 20% fluctuation in baseline *f*EPSPs were discarded. The *f*EPSP slope was measured using 7 data points between 10% and the 90% of maximum amplitude of the *f*EPSP waveform (Fig. 1A, right panel) using the equation:

$$S_N = \frac{R_{t10\%} - R_{t90\%}}{t_{10\%} - t_{90\%}}$$

where S_N = slope (μV/ms), R = response (μV), t = time (ms).

Once the slice response stabilized, an additional 10 min of *f*EPSPs slopes were recorded to calculate the mean baseline *f*EPSP slope (baseline period), which was then normalized as 100%. Perfusion of 100 μM picrotoxin (Sigma–Aldrich, St. Louis, MO)

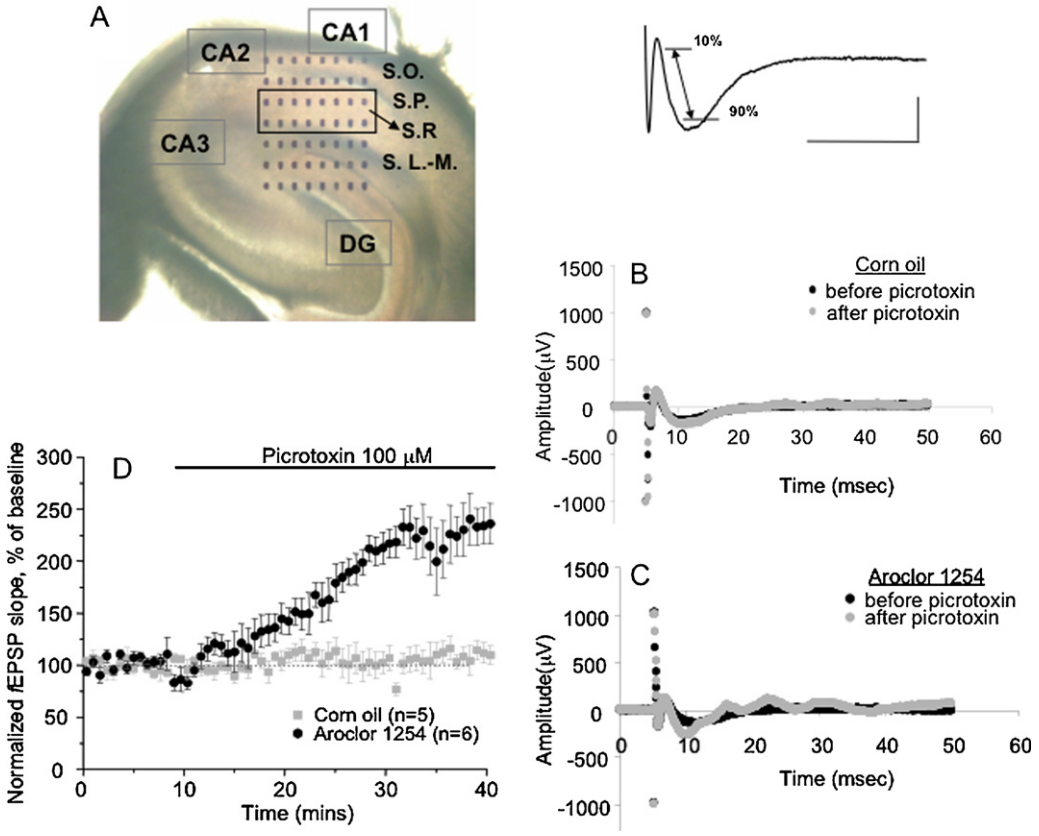

Fig. 1. Developmental exposure to PCB mixture Aroclor 1254 enhances excitotoxicity to picrotoxin *ex vivo*. (A) Med-64 multielectrode array (MEA) positioned on a hippocampal slice (left panel) and a depiction of a waveform of a field excitatory postsynaptic potential (*f*EPSP) indicating the location where slope was measured (right panel). Abbreviation: CA, Cornu Ammon; DG, Dentate Gyrus; S.O., Stratum Oriens; S.T., Stratum Pyramidale; S.R., Stratum Radiatum (the region indicated by the box); S. L.-M.; Stratum Lucidum-Moleculare. Scale: 200 μV, 10 ms. (B) Representative *f*EPSP recorded from a hippocampal slice prepared from a control rat before (at 10 min) and 30 min after the addition of picrotoxin (100 μM) to the aCSF. (C) Representative *f*EPSP recorded from a hippocampal slice prepared from a rat exposed during the perinatal period to Aroclor 1254, before (at 10 min) and after (at 40 min) addition of picrotoxin (100 μM) in the aCSF. Note the prominent after-discharges observed with all hippocampi prepared from the Aroclor 1254-exposed animals after perfusion of picrotoxin. (D) Normalized *f*EPSP slopes recorded from control (n = 5) and Aroclor 1254 treated animals (n = 6) before and after inclusion of picrotoxin in aCSF perfusate. *f*EPSP were recorded in response to single biphasic stimuli to Schaffer-Collateral/Commissural pathway of CA1 at 0.05 Hz. Data shown are the means ± S.E.